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- (71) Applicant (*for all designated States except US*): EISA CO., LTD. [JP/JP]; 6-10, Koishikawa 4-chome, Bunkyo-ku, Tokyo 112-8088 (JP).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): HATA, Katsura [JP/JP]; 20-6, Matsushiro 2-chome, Tsukuba-shi, Ibaraki 305-0035 (JP). OGAWA, Kaoru [JP/JP]; 102, 16-18, Kasuga 4-chome, Tsukuba-shi, Ibaraki 305-0821 (JP). TSUKADA, Itaru [JP/JP]; 7-4, Sakaecho 6-chome, Ushiku-shi, Ibaraki 300-1233 (JP). NAKAMOTO, Kazutaka [JP/JP]; 402, 15-8, Azuma 3-chome, Tsukuba-shi, Ibaraki 305-0031 (JP). SAGANE, Koji [JP/JP]; 303, 2-9, Amakubo 2-chome, Tsukuba-shi, Ibaraki 300-0005 (JP). TANAKA, Keigo [JP/JP]; 9-9, Matsushiro 2-chome, Tsukuba-shi, Ibaraki 305-0035 (JP). TSUKAHARA, Kappei [JP/JP]; 4-24, Ninomiya 4-chome, Tsukuba-shi, Ibaraki 305-0051 (JP). HORII, Toshihiro [JP/JP]; 19-2, Midorigaoka 3-chome, Toyonaka-shi, Osaka 560-0002 (JP).
- (74) Agents: SHIMIZU, Hatsushi et al.; Kantetsu Tsukuba Bldg. 6F, 1-1-1, Oroshi-machi, Tsuchiura-shi, Ibaraki 300-0847 (JP).
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(54) Title: METHODS OF SCREENING FOR COMPOUNDS THAT INHIBIT THE BIOSYNTHESIS OF GPI IN MALARIA PARASITES

(57) Abstract: The present inventors succeeded in isolating GWT1 (PfGWT1), which is one of the enzymes involved in GPI biosynthesis in the malaria parasite *P. falciparum*. In addition, the inventors revealed that degenerate mutant DNAs, with a lower AT content than the DNA encoding the PfGWT1 protein, can complement the phenotype of GWT1-deficient yeast. Based on the findings, the present invention provides the GWT1 protein of malaria parasites and the use of the protein in methods of screening for antimalarial drugs. The present invention also provides degenerate mutant DNAs encoding proteins involved in GPI biosynthesis, and which have a lower AT content than the original DNAs. The present invention also provides methods of screening for antimalarial drugs which use the degenerate mutant DNAs.

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DESCRIPTION

METHODS OF SCREENING FOR COMPOUNDS THAT INHIBIT THE BIOSYNTHESIS OF
GPI IN MALARIA PARASITESTechnical Field

The present invention relates to methods of screening for compounds that inhibit the biosynthesis of GPI in malaria parasites.

Background Art

Malaria is the most common infectious human disease caused by parasitic protozoans. The disease is caused by infection with malaria parasites and is mediated by the mosquito, *Anopheles gambiae*. Every year there are estimated 500 million cases of malaria infection, including more than two million fatal cases (Gardner, et al., Nature 419:498-511, 2003). At present 40% of the world's population lives in malaria-epidemic areas, where it is said that one in every three infants dies from malaria.

Glycosylphosphatidylinositol (GPI) is known to play a key role in the growth and infectivity of parasites, including malaria parasites. There are many GPI-anchored proteins on the cell surface of these parasites. GPI-anchored proteins include proteins such as MSP-1 that function when the parasites invade red blood cells. GPI proteins act as parasitic antigens and initiate an immune response in the host. Thus, they have long been the subject of research aimed at vaccine development.

GPI not only functions as an anchor to tether proteins to the cell membrane, but is also an abundant glycolipid component of malaria parasite cell membranes. Recent studies have revealed that GPI is toxic and causes lethal symptoms. GPI induces the expression of inflammatory cytokines such as TNF- α , and of adhesion molecules. As a result, infected red blood cells adhere to capillaries, obstructing vessels (sequestration), brain blood vessels in particular. This induces further inflammatory reactions that are believed to lead to encephalopathy. Very recently, Schofield et al. reported that an anti-GPI antibody reduces lethality in an *in vivo* infection model

system using the murine malaria parasite *Plasmodium berghei*, and that *in vitro*, the antibody inhibits late inflammatory reactions caused by *Plasmodium falciparum* (Schofield L, et al., Nature 418:785-789, 2002). These findings suggest that GPI is a major factor in the lethality of malarial infections.

It has also been reported that the acylation of inositol is essential for binding mannose to GPI (Gerold, P. et al., Biochem. J. 344:731-738, 1999), and that the inhibition of inositol acylation, caused by excess glucosamine, inhibits the maturation of the malaria parasite *P. falciparum* (Naik, R. S. et al., J. Biol. Chem. 278:2036-2042, 2003). Thus, compounds that can selectively inhibit GPI biosynthesis, particularly the acylation of inositol, may be highly useful antimalarial drugs.

Disclosure of the Invention

An objective of the present invention is to provide antimalarial drugs that inhibit the biosynthesis of GPI. More specifically, the present invention provides the malaria parasite DNA that encodes the GWT1 protein, which is a protein involved in the biosynthesis of GPI (GPI synthase). The present invention also provides a method of using this DNA in methods of screening for antimalarial drugs. The present invention also provides degenerate mutant DNAs of the DNA that encodes the malaria parasite GPI biosynthesis protein. These degenerate mutant DNAs have a lower AT content than the original DNA. The present invention also provides a method of using the degenerate mutant DNAs in methods of screening for antimalarial drugs.

The GWT1 gene was originally found to encode a fungal GPI-anchored protein synthase (WO 02/04626), and is conserved in organisms ranging from yeasts to humans. The present inventors confirmed that GWT1 homologues (PfGWT1 for *P. falciparum* GWT1; PyGWT1 for *P. yoelii yoelii* GWT1) are included in the entire genomic sequences of *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium yoelii yoelii* (*P. yoelii yoelii*) (Gardner, et al., Nature 419:498-511, 2003; Carlton et al., Nature 419:512-519, 2003). The present inventors also found that the GWT1 gene product acts as a GlcN-PI acyltransferase in the GPI biosynthesis pathway. The PfGWT1 gene product is expected

to have similar activity, and thus compounds that inhibit this activity can be promising antimalarial drugs.

In WO 02/04626, the present inventors disclosed a group of compounds that inhibit the activity of the fungal GWT1 gene product. Compounds inhibiting the activity of the PfGWT1 gene product were expected to be antimalarial drugs.

In the present invention, the present inventors succeeded in isolating a region thought to be almost the full length of the PfGWT1. Using the GWT1 gene products of malaria parasites such as *P. falciparum*, antimalarial drugs can be screened through binding assays, glucosaminyl(acyl)phosphatidylinositol (PI-GlcN) acyltransferase assays, or using GPI-anchored protein detection systems. Compounds obtained from such screenings can be promising antimalarial drugs. Furthermore, the present inventors revealed that degenerate mutant DNAs (degenerate mutants of the DNA that encodes the malaria parasite GPI biosynthesis protein) having a lower AT content than the original DNA, complement the phenotype of the GWT1 gene-deficient fungus. Thus, it is possible to screen for compounds that inhibit the activity of proteins involved in GPI biosynthesis in malarial parasites by using, as an index, the phenotype of a GPI synthase gene-deficient fungus, into which a degenerate mutant DNA with a lower AT content (than the DNA encoding the GPI biosynthesis protein in malaria parasites) has been introduced.

Specifically, the present invention provides the following [1] to [25]:

[1] a DNA according to any one of (a) to (d), which encodes a protein of a malaria parasite having a GlcN-PI acyltransferase activity:

(a) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2 or 4,

(b) a DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3,

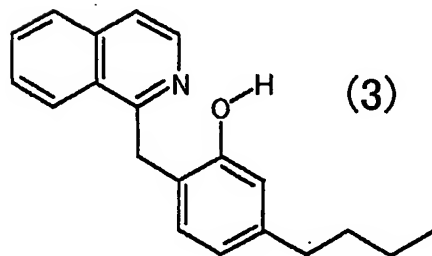
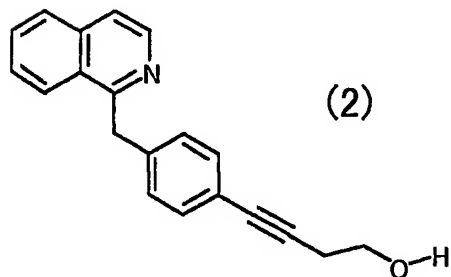
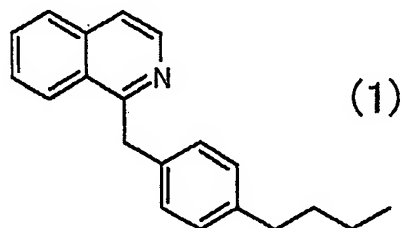
(c) a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3 under stringent conditions, and

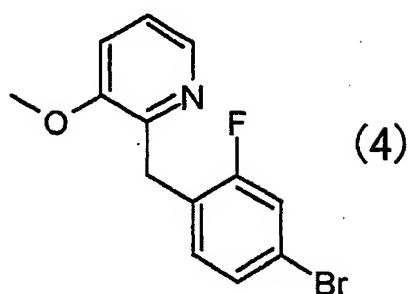
(d) a DNA encoding a protein which comprises the amino acid sequence of SEQ ID NO: 2 or 4, in which one or more amino acids have been added, deleted, substituted, and/or inserted;

[2] a protein encoded by the DNA according to [1];
[3] a vector into which the DNA according to [1] is inserted;
[4] a transformant which retains, in an expressible state, the DNA according to [1] or the vector according to [3] ;

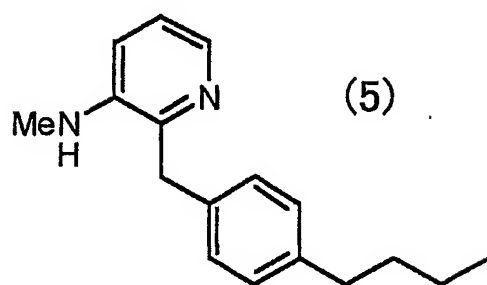
[5] an antimalarial drug which comprises as an active ingredient a compound that inhibits the activity of the protein according to [2];

[6] the antimalarial drug according to [5], wherein the compound that inhibits the activity of the protein according to [2] is at least one selected from the group consisting of the following compounds (1) to (5):





and,



[7] a method of screening for a compound having antimalarial activity, which comprises the steps of:

- (1) contacting the protein according to [2] with a test sample and a labeled compound that has the activity of binding to the protein,
- (2) detecting the labeled compound that binds to the protein, and,
- (3) selecting a test sample that decreases the amount of labeled compound that binds to the protein;

[8] the method according to [7], wherein the labeled compound that has the activity of binding to the protein is produced by labeling at least one compound selected from the group consisting of the compounds (1) to (5) according to [6];

[9] a method of screening for a compound having antimalarial activity, which comprises the steps of:

- (1) contacting a test sample with the protein according to [2],
- (2) detecting GlcN-(acyl)PI, and,
- (3) selecting a test compound that decreases the level of GlcN-(acyl)PI;

[10] a method of screening for a compound having antimalarial activity, which comprises the steps of:

- (1) contacting a test sample with a cell overexpressing the protein

according to [2],

(2) determining the amount of GPI-anchored protein transported to the cell wall, and,

(3) selecting a test sample that decreases the amount of the GPI-anchored protein transported to the cell wall, as determined in step (2);

[11] a method for treating malaria, which comprises administering a compound that inhibits the activity of the protein according to [2];

[12] the method according to [11], wherein the compound that inhibits the activity of the protein according to [2] is the compound according to [5];

[13] a DNA encoding a protein that has the activity of complementing the phenotype of a GPI synthase gene-deficient yeast, which is a degenerate mutant of a DNA encoding a protein involved in GPI biosynthesis in malaria parasites, and that has a lower AT content than the original DNA;

[14] a DNA encoding a protein that has the activity of complementing the phenotype of a GPI synthase gene-deficient yeast, which is a degenerate mutant of a DNA encoding a protein involved in GPI biosynthesis in malaria parasites, and that has an AT content which is reduced by 70%;

[15] the DNA according to [13] or [14], which is selected from the group consisting of:

(a) a DNA encoding a protein that comprises any one of the amino acid sequences of SEQ ID NOs: 2 and 4, and odd sequence identification numbers in SEQ ID NOs: 6-47,

(b) a DNA comprising any one of the nucleotide sequences of SEQ ID NOs: 1 and 3, and even sequence identification numbers in - SEQ ID NOs: 6-47,

(c) a DNA hybridizing under stringent conditions to a DNA that comprises any one of the nucleotide sequences of SEQ ID NOs: 1 and 3, and even sequence identification numbers in SEQ ID NOs: 6-47, and,

(d) a DNA encoding a protein which comprises any one of the amino acid sequences of SEQ ID NOs: 2 and 4, and odd sequence identification numbers in SEQ ID NOs: 6-47, in which one or more amino acids have

been added, deleted, substituted, and/or inserted;

[16] a DNA comprising the nucleotide sequence of SEQ ID NO: 5;

[17] a vector in which a DNA according to any one of [13] to [16] is inserted;

[18] a transformant which retains, in an expressible state, the DNA according to any one of [13] to [16] or the vector according to [17];

[19] the transformant according to [18], which is a GPI synthase gene-deficient fungus;

[20] the transformant according to [18], which is a GPI synthase gene-deficient yeast;

[21] a method for producing a protein encoded by a DNA according to any one of [13] to [16], which comprises the steps of culturing the transformant according to any one of [18] to [20], and recovering the expressed protein from the transformant or the culture supernatant;

[22] a method of screening for a compound having antimalarial activity, which comprises the steps of:

(1) contacting a test sample with a GPI synthase gene-deficient fungus expressing the DNA according to any one of [13] to [16],

(2) assaying the growth of that fungus, and,

(3) selecting a test compound that inhibits the growth of that fungus;

[23] a method of screening for a compound having antimalarial activity, which comprises the steps of:

(1) contacting a test sample with a GPI synthase gene-deficient fungus expressing the DNA according to any one of [13] to [16],

(2) determining the amount of a GPI-anchored protein transported to the fungal cell walls, and,

(3) selecting a test sample that decreases the amount of the GPI-anchored protein transported to the cell wall, as determined in step (2);

[24] a method of screening for a compound having antimalarial activity, which comprises the steps of:

(1) introducing the DNA according to any one of [13] to [16] into a GPI synthase gene-deficient fungus and expressing the protein

encoded by the DNA,

- (2) preparing the protein expressed in step (1),
- (3) contacting the prepared protein with a test sample and a labeled compound that has the activity of binding to the protein,
- (4) detecting the labeled compound that binds to the protein, and,
- (5) selecting a test sample that decreases the amount of labeled compound that binds to the protein; and,

[25] a method of screening for a compound having antimalarial activity, which comprises the steps of:

- (1) introducing into a GWT1-deficient fungus, (i) a DNA encoding a protein that has the activity of complementing the phenotype of a GWT1-deficient yeast, wherein the DNA is a degenerate mutant of a DNA encoding a malaria parasite GWT1 protein that has a lower AT content than the original DNA, or (ii) a vector into which the degenerate mutant of DNA has been inserted, and expressing the protein encoded by the degenerate mutant DNA,
- (2) preparing the protein expressed in step (1),
- (3) contacting the prepared protein with a test sample,
- (4) detecting GlcN-(acyl)PI, and
- (5) selecting a test compound that decreases the level of GlcN-(acyl)PI.

The DNA encoding the GWT1 protein of *Plasmodium falciparum* (PfGWT1) was isolated for the first time in the present invention. The nucleotide sequence of the DNA encoding the PfGWT1 protein is shown in SEQ ID NO: 1, and the amino acid sequence of the PfGWT1 protein is set forth in SEQ ID NO: 2. In addition, the nucleotide sequence of the DNA encoding the GWT1 protein of *Plasmodium vivax* (PvGWT1) is shown in SEQ ID NO: 3, and the amino acid sequence of the PvGWT1 protein is set forth in SEQ ID NO: 4.

The GWT1 protein is involved in the biosynthesis of glycosylphosphatidylinositol (GPI), which is essential for the growth and infectivity of malaria parasites. Thus, compounds that inhibit the activity of the malaria parasite GWT1 protein can be used as antimalarial drugs. Such antimalarial drugs can be screened using this malaria parasite GWT1 protein.

The present invention provides DNAs encoding the malaria parasite GWT1 protein. Such DNAs include DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2 or 4, and DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3.

The present invention also provides DNAs encoding proteins that are functionally equivalent to the protein comprising the amino acid sequence of SEQ ID NO: 2 or 4. Herein, the expression "functionally equivalent" refers to having biological properties equivalent to those of the protein of interest, comprising the amino acid sequence of SEQ ID NO: 2 or 4 (the PfGWT1 or PvGWT1 proteins). The biological properties of the PfGWT1 and PvGWT1 proteins include GlcN-PI acyltransferase activity. The GlcN-PI acyltransferase activity can be measured by the method reported by Costello and Orlean (J. Biol. Chem. (1992) 267:8599-8603), or Franzot and Doering (Biochem. J. (1999) 340:25-32).

DNAs encoding proteins functionally equivalent to the protein comprising the amino acid sequence of SEQ ID NO: 2 or 4 include: DNAs that hybridize under stringent conditions to the DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3, and DNA encoding a protein which comprises the amino acid sequence of SEQ ID NO: 2 or 4, in which one or more amino acids have been added, deleted, substituted, and/or inserted.

The DNAs of the present invention can be isolated by methods well known to those skilled in the art. Examples of such methods include the use of hybridization (Southern E.M., J. Mol. Biol. 98: 503-517, 1975) and the polymerase chain reaction (PCR) (Saiki R.K. et al., Science 230: 1350-1354, 1985; Saiki R.K. et al., Science 239: 487-491, 1988). More specifically, it would be routine experimentation for those skilled in the art to isolate, from malaria parasites, a DNA highly homologous to DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3, using the DNA of SEQ ID NO: 1 or 3 or portions thereof as a probe, or by using as a primer a DNA which specifically hybridizes to the DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3. Furthermore, DNAs that can be isolated by hybridization or PCR techniques, and that hybridize with the DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3, are also comprised in the DNAs of the

present invention. Such DNAs include DNA encoding a malaria parasite homologue of the protein comprising the amino acid sequence of SEQ ID NO: 2 or 4. The malaria parasite homologue includes those of *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*, which comprise the amino acid sequence of SEQ ID NO: 2 or 4.

Preferably, a DNA described above is isolated using hybridization reactions under stringent hybridization conditions. As used herein, the expression "stringent hybridization conditions" refers to, for example, hybridization in 4x SSC at 65°C followed by washing in 0.1x SSC at 65°C for one hour. Alternative stringent conditions are hybridization in 4x SSC containing 50% formamide at 42°C. Further alternative stringent conditions are hybridization in PerfectHyb™ (TOYOBO) solution at 65°C for 2.5 hours, followed by washing: (1) in 2x SSC containing 0.05% SDS at 25°C for five minutes; (2) in 2x SSC containing 0.05% SDS at 25°C for 15 minutes; and (3) in 0.1x SSC containing 0.1% SDS at 50°C for 20 minutes. The DNA thus isolated is expected to encode a polypeptide with a high homology at the amino acid level to the amino acid sequence of SEQ ID NO: 2 or 4. Herein, "high homology" means a sequence identity of at least 70% or more, preferably 80% or more, more preferably 90% or more, and most preferably 95% or more, in the whole amino acid sequence.

The degree of identity at the amino acid sequence level or nucleotide sequence level can be determined using the BLAST algorithm of Karlin and Altschul (Karlin S. and Altschul S.F, Proc. Natl. Acad. Sci. USA. 87: 2264-2268, 1990; Karlin S. and Altschul S.F, Proc. Natl. Acad. Sci. USA. 90: 5873-5877, 1993). BLAST algorithm-based programs, called BLASTN and BLASTX, have been developed (Altschul S.F. et al., J. Mol. Biol. 215: 403, 1990). When a nucleotide sequence is analyzed using BLASTN, the parameters are set, for example, at score= 100 and word length= 12. On the other hand, when an amino acid sequence is analyzed using BLASTX, the parameters are set, for example, at score= 50 and word length= 3. When the BLAST and Gapped BLAST programs are used, the default parameters for each program are used. Specific procedures for such analysis are known (please see the web site of the National Institute of Biotechnology Information

<http://www.ncbi.nlm.nih.gov>).

DNAs of the present invention comprise genomic DNAs, cDNAs, and chemically synthesized DNAs. A Genomic DNA or cDNA can be prepared according to conventional methods known to those skilled in the art. For example, a genomic DNA can be prepared as follows: (i) extracting a genomic DNA from malaria parasites; (ii) constructing a genomic library (using, for example, a plasmid, phage, cosmid, BAC, or PAC, as a vector); (iii) spreading the library; and then (iv) conducting colony hybridization or plaque hybridization using probes prepared based on a DNA which encodes the malaria parasite GWT1 protein of the present invention (e.g., SEQ ID NO: 1 or 3). Alternatively, genomic DNA can be prepared by PCR, using primers specific to a DNA which encodes the malaria parasite GWT1 protein of the present invention (e.g., SEQ ID NO: 1 or 3). On the other hand, cDNA can be prepared, for example, as follows: (i) synthesizing cDNA based on mRNA extracted from malaria parasites; (ii) constructing a cDNA library by inserting the synthesized cDNA into vectors such as λ ZAP; (iii) spreading the cDNA library; and (iv) conducting colony hybridization or plaque hybridization as described above. Alternatively, the cDNA can also be prepared using PCR.

The present invention also provides DNAs encoding proteins structurally similar to the protein comprising the amino acid sequence of SEQ ID NO: 2 or 4. Such DNAs include those which comprise nucleotide sequences encoding proteins comprising amino acid sequences in which one or more amino acid residues are substituted, deleted, inserted, and/or added. There is no limitation on the number and site of the amino acid mutation in proteins mentioned above, so long as the mutated protein retains functions of the original protein such as those described in Mark, D. F. et al., Proc. Natl. Acad. Sci. USA (1984) 81, 5662-5666; Zoller, M. J. & Smith, M., Nucleic Acids Research (1982) 10, 6487-6500; Wang, A. et al., Science 224, 1431-1433; Dalbadie-McFarland, G. et al., Proc. Natl. Acad. Sci. USA (1982) 79, 6409-6413. The percentage of mutated amino acids is typically 10% or less, preferably 5% or less, and more preferably 1% or less of the total amino acid residues. In addition, the number of mutated amino acids is usually 30 amino acids or less, preferably 15 amino

acids or less, more preferably five amino acids or less, still more preferably three amino acids or less, even more preferably two amino acids or less.

It is preferable that the mutant amino acid residue be one that retains the properties of the side-chain after its mutation (a process known as conservative amino acid substitution). Examples of amino acid side chain properties are hydrophobicity (A, I, L, M, F, P, W, Y, V) and hydrophilicity (R, D, N, C, E, Q, G, H, K, S, T). Side chains include: aliphatic side-chains (G, A, V, L, I, P); side chains containing an hydroxyl group (S, T, Y); side chains containing a sulfur atom (C, M); side chains containing a carboxylic acid and an amide (D, N, E, Q); basic side-chains (R, K, H); and aromatic side-chains (H, F, Y, W).

A fusion protein comprising the malaria parasite GWT1 protein is an example of a protein to which one or more amino acids residues have been added. Fusion proteins can be made by techniques well known to a person skilled in the art. For example, and without limitation to this particular technique, the DNA encoding the malaria parasite GWT1 protein of the present invention can be combined with DNA encoding another peptide or protein such that their reading frames match. A protein of the present invention can form a fusion protein with a number of known peptides. Such peptides include FLAG (Hopp, T. P. *et al.*, *Biotechnology* (1988) 6, 1204-1210), 6x His, 10x His, Influenza agglutinin (HA), human c-myc fragment, VSP-GP fragment, p18HIV fragment, T7-tag, HSV-tag, E-tag, SV40T antigen fragment, lck tag, α -tubulin fragment, B-tag, and Protein C fragment. Examples of proteins that may be fused to a protein of the present invention include glutathione-S-transferase (GST), HA, immunoglobulin constant region, β -galactosidase, and maltose-binding protein (MBP).

In addition to using the above-mentioned hybridization and PCR techniques, those skilled in the art could prepare the above-described DNA by methods including, for example, site-directed mutagenesis to introduce mutations in that DNA (Kramer W. and Fritz H-J., *Methods Enzymol.* 154: 350, 1987). A protein's amino acid sequence may also be mutated in nature due to mutation of the nucleotide sequence which encodes the protein. In addition, degenerate mutant DNAs, in which

nucleotide mutations do not result in amino acid mutations in the proteins (degeneracy mutants), are also comprised in the present invention. Furthermore, the present invention also comprises proteins encoded by the above-described DNAs of this invention.

The present invention provides vectors containing the DNAs of the present invention, transformants retaining the DNAs or vectors of the present invention, and methods for producing proteins of the present invention which utilize these transformants.

A vector of the present invention is not limited so long as the DNA inserted into the vector is stably retained. For example, pBluescript® vector (Stratagene) is preferable as a cloning vector when using *E. coli* as a host. An expression vector is particularly useful when using a vector to produce a protein of the present invention. The expression vector is not specifically limited, so long as it expresses proteins *in vitro*, in *E. coli*, in cultured cells, and *in vivo*. Preferable examples of expression vectors include the pBEST vector (Promega Corporation) for *in vitro* expression, the pET vector (Novagen) for expression in *E. coli*, the pME18S-FL3 vector (GenBank Accession No. AB009864) for expression in cultured cells, and the pME18S vector (Mol. Cell Biol. 8: 466-472, 1988) for *in vivo* expression. The insertion of a DNA of the present invention into a vector can be carried out by conventional methods, for example, by a ligase reaction using restriction enzyme sites (Current Protocols in Molecular Biology, ed. by Ausubel et al., John Wiley & Sons, Inc. 1987, Section 11.4-11.11).

The host cell into which the vector of the present invention is introduced is not specifically limited, and various host cells can be used according to the objectives of this invention. For example, cells that can be used to express the proteins include, but are not limited to, bacterial cells (e.g., *Streptococcus*, *Staphylococcus*, *E. coli*, *Streptomyces*, *Bacillus subtilis*), fungal cells (e.g., yeast, *Aspergillus*), insect cells (e.g., *Drosophila* S2, *Spodoptera* SF9), animal cells (e.g., CHO, COS, HeLa, C127, 3T3, BHK, HEK293, Bowes melanoma cell), and plant cells. The transfection of a vector to a host cell can be carried out by conventional methods such as calcium phosphate precipitation, electroporation (Current protocols in

Molecular Biology, ed. by Ausubel et al., John Wiley & Sons, Inc. 1987, Section 9.1-9.9), the Lipofectamine method (GIBCO-BRL), and microinjection.

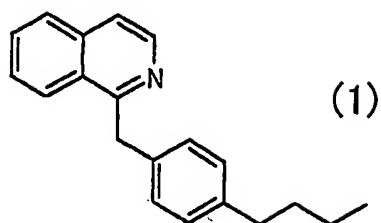
By incorporating an appropriate secretion signal into the protein of interest, the protein expressed in host cells can be secreted into the lumen of the endoplasmic reticulum, into cavities around the cells, or into the extracellular environment. These signals may be endogenous or exogenous to the protein of interest.

When a protein of the present invention is secreted into the culture medium, it is collected from that medium. If a protein of the present invention is produced intracellularly, the cells are lysed and then the protein is collected.

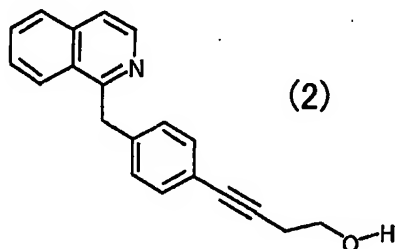
A protein of the present invention can be collected and purified from a recombinant cell culture using methods known in the art, including, but not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anionic or cationic exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography.

Compounds including DNAs of the present invention are isolated compounds. Herein, the term "isolated" refers to being separated from the original environment (for example, the natural environment if it is naturally-occurring). A compound in a sample where the compound of interest is substantially abundant, and/or in a sample where the compound of interest has been partially or substantially purified, is an "isolated" compound. The term "substantially purified", as used herein, refers to a state where the compound has been separated from the original environment, and from which at least 60%, preferably 75%, and most preferably 90% of other coexisting natural components have been removed.

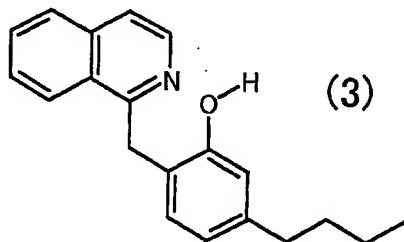
The present invention provides an antimalarial drug that inhibits the activity of the GWT1 gene product of malaria parasites. A preferred compound inhibiting the activity of the GWT1 gene product of malaria parasites is the compound described in WO 02/04626, and includes the compounds (1) to (5):



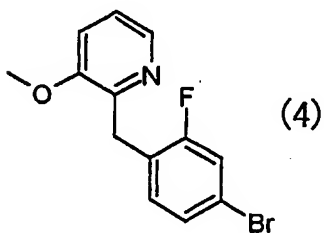
compound (1): 1-(4-butyl benzyl) isoquinoline



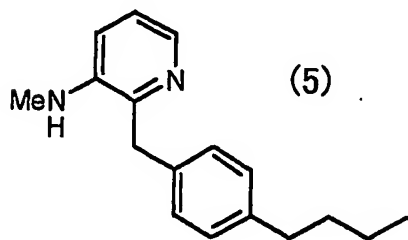
compound (2): 4-[4-(1-isoquinolyl methyl) phenyl]-3-butyne-1-ol



compound (3): 5-butyl-2-(1-isoquinolyl methyl) phenol



compound (4): 2-(4-bromo-2-fluorobenzyl)-3-methoxypyridine



compound (5): N-[2-(4-butyl benzyl)-3-pyridyl]-N-methylamine

A Compound that inhibits the activity of the malaria parasite GWT1 gene product, or a salt thereof, or a hydrate thereof, can be administered as it is to mammals (preferably humans). It can also be formulated by a conventional method into a tablet, powder, fine granule, granule, coated tablet, capsule, syrup, troche, inhalant, suppository, injection, ointment, eye ointment, eye drop, nasal drop, ear drop, cataplasm, lotion, and such, and then administered.

For formulation of a pharmaceutical, auxiliary agents ordinarily used in pharmaceutical formulations (for example, fillers, binders, lubricants, coloring agents, flavoring agents, and as necessary, stabilizers, emulsifiers, absorbefacient, surfactants, pH regulators, antiseptics, and antioxidants) can be used. A pharmaceutical formulation can be prepared using an ordinary method combining components that are generally used as ingredients for pharmaceutical preparations.

For example, oral formulations can be produced by combining a compound of the present invention or a pharmaceutically acceptable salt thereof with a filler, and as necessary, a binder, disintegrator, lubricant, coloring agent, flavoring agent, and such, and then formulating the mixture into a powder, fine granule, granule, tablet, coated tablet, capsule, and such by usual methods.

Examples of these components include: animal fat and vegetable oils such as soybean oil, beef tallow, and synthetic glyceride; hydrocarbons such as liquid paraffin, squalene, and solid paraffin; ester oils such as octyldodecyl myristate and isopropyl myristate; higher alcohols such as cetostearyl alcohol and behenyl alcohol; silicone resin; silicone oil; surfactants such as polyoxyethylene fatty acid ester, sorbitan fatty acid ester, glycerol fatty acid ester, polyoxyethylene sorbitan fatty acid ester, polyoxyethylene hardened castor oil, and polyoxyethylene polyoxypropylene block copolymer; water-soluble macromolecules such as hydroxyethyl cellulose, polyacrylic acid, carboxyvinyl polymer, polyethylene glycol, polyvinyl pyrrolidone, and methyl cellulose; lower alcohols such as ethanol and isopropanol; polyhydric alcohols such as glycerol, propylene glycol, dipropylene glycol, and sorbitol; sugars such as glucose and sucrose; inorganic powder such as silicic acid anhydride,

magnesium aluminum silicate, and aluminum silicate; and purified water. Examples of fillers include lactose, corn starch, refined white sugar, glucose, mannitol, sorbitol, crystalline cellulose, and silicon dioxide. Binders are polyvinyl alcohol, polyvinyl ether, methyl cellulose, ethyl cellulose, gum arabic, tragacanth, gelatin, shellac, hydroxypropylmethyl cellulose, hydroxypropyl cellulose, polyvinyl pyrrolidone, polypropyleneglycol polyoxyethylene block polymer, meglumine, and such. Examples of disintegrators include starch, agar, powdered gelatin, crystalline cellulose, calcium carbonate, sodium hydrogencarbonate, calcium citrate, dextrin, pectin, and calcium carboxymethylcellulose. Lubricants are magnesium stearate, talc, polyethyleneglycol, silica, hardened vegetable oil, and such. Examples of coloring agents are those accepted for addition to pharmaceuticals. Flavoring agents are cocoa powder, l-menthol, aromatic dispersant, mint oil, borneol, cinnamon powder, and such. The use of sugar coating and other appropriate coating as necessary is of course permissible for these tablets and granules.

Furthermore, liquid formulations such as syrups and injections can be prepared using conventional methods. In such methods, pH regulators, solubilizers, isotonizing agents, and such, and as necessary solubilizing adjuvants, stabilizers, and so on, are added to the compounds of this invention or pharmaceutically acceptable salts thereof.

Methods for producing external formulations is not restricted and can be a conventional method. That is, base materials used for formulation can be selected from various materials ordinarily used for medicaments, quasi-drugs, cosmetics, and such. Specifically, the base materials to be used are, for example, animal fat and vegetable oils, mineral oils, ester oils, waxes, higher alcohols, fatty acids, silicone oils, surfactants, phospholipids, alcohols, polyhydric alcohols, water soluble macromolecules, clay minerals, and purified water. As necessary, pH regulators, antioxidants, chelating agents, antiseptic and antifungal agents, coloring matters, fragrances, and such may also be added. However the base materials of the external formulations of the present invention are not limited thereto. Furthermore, as necessary, components such as those that

have a differentiation-inducing effect, blood flow accelerants, fungicides, antiphlogistic agents, cell activators, vitamins, amino acids, humectants, and keratolytic agents can be combined. The above-mentioned base materials are added in an amount that leads to the concentration usually used for external formulations.

The term "salt" as described in the present invention preferably includes, for example, a salt with an inorganic or organic acid, a salt with an inorganic or inorganic base, or a salt with an acidic or basic amino acid. In particular, a pharmaceutically acceptable salt is preferable. Acids and bases form salts at an appropriate ratio of 0.1 to 5 molecules of acid or base to one molecule of the compound.

Preferable examples of a salt with an inorganic acid are a salt with hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, and phosphoric acid. Preferably, a salt with an organic acid includes a salt with acetic acid, succinic acid, fumaric acid, maleic acid, tartaric acid, citric acid, lactic acid, stearic acid, benzoic acid, methanesulfonic acid, and p-toluenesulfonic acid.

Preferable examples of a salt with an inorganic base are: an alkali metal salt such as a sodium salt and a potassium salt; an alkaline earth metal salt such as a calcium salt and a magnesium salt; an aluminum salt, and an ammonium salt. Preferably, a salt with an organic base includes a salt with diethylamine, diethanolamine, meglumine, and N,N'-dibenzylethylenediamine.

Preferable examples of a salt with an acidic amino acid are a salt with aspartic acid and glutamic acid, and preferably, a salt with a basic amino acid includes a salt with arginine, lysine, and ornithine.

The compounds of the present invention or salts thereof, or hydrates thereof can be administered orally or parenterally by a conventional method without limitation as to their form. They can be formulated into dosage forms such as tablets, powders, fine granules, capsules, syrups, troches, inhalants, suppositories, injections, ointments, eye ointments, eye drops, nasal drops, ear drops, cataplasms, and lotions. The dose of the pharmaceutical compositions of this invention can be selected appropriately

depending on the degree of the symptoms, the patient's age, sex and weight, the dosage form, the type of salt, the specific type of disease, and such.

Compounds of the present invention are administered to a patient in a therapeutically effective dose. Herein, "therapeutically effective dose" refers to the amount of pharmaceutical agent that yields the desired pharmacological result and is effective in the recovery or relief from the symptoms of the patient to be treated. The dose differs markedly depending on the type of disease, the degree of symptoms, the patient's weight, age, sex, sensitivity to the agent. However, the normal adult dosage for one day is about 0.03 mg to 1000 mg, preferably 0.1 mg to 500 mg, more preferably 0.1 mg to 100 mg, when administered from once to several times a day, or from once to several times over several days. The dose for injections is normally, about 1 to 3000 $\mu\text{g}/\text{kg}$, and is preferably about 3 to 1000 $\mu\text{g}/\text{kg}$.

In addition, the present invention relates to a method of screening for antimalarial drugs using the malaria parasite GWT1 gene product. Such a screening method includes, but is not limited to: [1] A binding assay which screens for compounds that compete with a labeled compound to bind with the malaria parasite GWT1 gene product; [2] A GlcN-PI acyltransferase assay system to screen for compounds that inhibit the GlcN-PI acyltransferase activity of the malaria parasite GWT1 gene product; and [3] A GPI-anchored protein detection system in which the malaria parasite GWT1 gene product is expressed in cells, preferably fungal cells, and then the GPI-anchored proteins on the cell surface are detected. The present invention is not limited to these methods, and comprises any method of screening for antimalarial drugs using the malaria parasite GWT1 gene product. The methods [1] to [3] listed above are described below in detail.

[1] A binding assay to screen for compounds that compete with a labeled compound to bind with the malaria parasite GWT1 gene product

The two methods according to the present invention are disclosed below, namely (1) a method for preparing the malaria parasite GWT1 gene product (hereinafter referred to as the malaria parasite GWT1

protein) and (2) a method for a binding experiment involving a labeled compound (hereinafter referred to as a binding assay).

(1) Method for preparing the malaria parasite GWT1 protein

The malaria parasite GWT1 protein is prepared from a cell membrane fraction, preferably from fungal cells, more preferably from cells of *S. cerevisiae* into which the DNA encoding the malaria parasite GWT1 protein of SEQ ID NO: 2 has been introduced. It is preferable to introduce such a DNA into GWT1 gene-deficient cells. In the binding assay, the prepared membrane fraction may be used without any further treatment, or can be further purified before use. The procedure using *S. cerevisiae* is described below in detail.

(a) Introduction of the malaria parasite GWT1 gene

The malaria parasite GWT1 gene used in the present invention can be a naturally-occurring gene, or preferably, it can be synthesized based on the amino acid sequence of SEQ ID NO: 2 or 4. The malaria parasite GWT1 gene is very rich in adenine and thymine. Thus, it was predictable that the gene will be difficult to manipulate with ordinary gene recombination techniques, and that gene expression in yeast, cells, and such will be inefficient. Therefore, it is preferable to design a nucleotide sequence in which codons corresponding to each of the corresponding amino acids have been replaced with those that are thought to express efficiently in yeast, cells, and such, and conduct DNA synthesis based on this designed sequence to create an artificial malaria parasite GWT1 gene, which is then used in the experiments described below.

An expression plasmid for the malaria parasite GWT1 is prepared by inserting the malaria parasite GWT1 gene into an *S. cerevisiae* expression vector, for example, an expression vector prepared by inserting a suitable promoter and terminator, such as the pKT10-derived GAPDH promoter and GAPDH terminator, into the expression vector YEp352's multi-cloning site (Tanaka et al., Mol. Cell Biol., 10:4303-4313, 1990). *S. cerevisiae* (e.g., G2-10 strain) is cultured in an appropriate medium (e.g., YPD medium (Yeast extract-Polypeptone-Dextrose medium)) while shaking at an appropriate temperature (e.g., 30°C), and the cells are harvested during the late logarithmic growth phase. After washing, the

GWT1-expression plasmid is introduced into *S. cerevisiae* cells using, for example, the lithium-acetate method. This method is described in the User Manual of YEAST MAKER™ Yeast Transformation System (BD Biosciences Clontech). A malaria parasite GWT1-overexpressing strain and a strain carrying a negative control vector can be obtained by culturing the transformed cells on SD (ura-) medium at 30°C for two days.

Expression vectors and gene transfer methods for fungal species other than *S. cerevisiae* have been reported as follows: expression vectors such as pcL for *Schizosaccharomyces pombe* (*S. pombe*) and their transfer methods are described by Igarashi et al. (Nature 353:80-83, 1991); expression vectors such as pRM10 for *C. albicans* and their transfer methods are described by Pla J. et al. (Yeast, 12: 1677-1702, 1996); expression vectors such as pAN7-1 for *A. fumigatus* and their transfer methods are described by Punt P.J. et al. (GENE, 56: 117-124, 1987); and expression vectors such as pPM8 for *C. neoformans* and their transfer methods are described by Monden P. et al. (FEMS Microbiol. Lett., 187: 41-45, 2000).

(b) Method for preparing membrane fractions

S. cerevisiae cells in which the malaria parasite GWT1 gene has been introduced are cultured in an appropriate medium (e.g., SD (ura-) liquid medium) while being shaken at an appropriate temperature (e.g., 30°C). The fungal cells are harvested during the mid-logarithmic growth phase, washed, and then suspended in an appropriate amount (e.g., three times the volume of fungal cells) of homogenization buffer (e.g., 50 mM Tris-HCl, pH 7.5, 10 mM EDTA, Complete™ (Roche)). An appropriate amount of glass beads (e.g., four times the volume of fungal cells) is added to the suspension. The mixture is vortexed and then allowed to stand on ice. This operation is repeated several times to crush fungal cells.

One milliliter of the homogenization buffer is added to the resulting lysate. The mixture is centrifuged, for example at 2,500 rpm for five minutes, to precipitate the glass beads and uncrushed fungal cells. The supernatant is transferred to another tube. The tube is centrifuged, for example at 13,500 rpm for ten minutes, to

precipitate a membrane fraction (total membrane fraction) comprising organelles. The precipitate is suspended in 1 ml of binding buffer (e.g., 0.1 M Phosphate buffer, pH 7.0, 0.05% Tween 20, Complete™ (Roche)), and then centrifuged, for example, at 2,500 rpm for one minute to remove unsuspended material. The supernatant is then centrifuged, for example at 15,000 rpm for five minutes. The precipitate is resuspended in 150 to 650 µl of binding buffer to prepare a membrane fraction.

Membrane fractions can be prepared from fungal species other than *S. cerevisiae* using the method of Yoko-o et al. for *S. pombe* (Eur. J. Biochem. 257:630-637, 1998); the method of Sentandreu M et al. for *C. albicans* (J. Bacteriol., 180: 282-289, 1998); the method of Mouyna I et al. for *A. fumigatus* (J. Biol. Chem., 275: 14882-14889, 2000); and the method of Thompson JR et al. for *C. neoformans* (J. Bacteriol., 181: 444-453, 1999).

Alternatively, the malaria parasite GWT1 protein can be prepared by expressing an *E. coli*, insect and mammalian cell or the like in non-fungal cells.

When mammalian cells are used, the malaria parasite GWT1 gene is ligated with an over-expression vector containing, for example, the CMV promotor, and then introduced into the mammalian cells. Membrane fractions can then be prepared according to the method of Petaja-Repo et al. (J. Biol. Chem., 276:4416-23, 2001).

Insect cells expressing the malaria parasite GWT1 gene (e.g., Sf9 cells) can be prepared using, for example, a baculovirus expression kit such as the BAC-TO-BAC® Baculovirus Expression system (Invitrogen). Membrane fractions can then be prepared according to the method of Okamoto et al. (J. Biol. Chem., 276:742-751, 2001).

The malaria parasite GWT1 protein can be prepared from *E. coli* by, for example, ligating the malaria parasite GWT1 gene into an *E. coli* expression vector such as the pGEX vector (Pfizer Inc.), and introducing the construct into *E. coli* such as BL21.

(2) Binding assay methods

(a) Synthesis of labeled compound

The labeled compound is prepared from a compound that has been

confirmed to bind to GWT1 proteins. Any compound which can bind to GWT1 proteins can be used. The labeled compound is preferably prepared from the compound described in WO 02/04626, more preferably from compounds according to (1) to (5) described above.

Any labeling method can be used. Preferably, the compound is labeled with a radioisotope, more preferably with ^3H . The radiolabeled compound can be prepared by typical production methods using a radioactive compound as a starting material. Alternatively, ^3H labeling can be achieved using an ^3H exchange reaction.

(b) Confirmation of specific binding

The labeled compound is added to the prepared membrane fraction and the mixture is allowed to stand on ice for an appropriate time, for example, one to two hours, while the binding reaction between the labeled compound and the membrane fraction takes place. The membrane fraction is precipitated by centrifuging the mixture, for example at 15,000 rpm for three minutes. The precipitate is resuspended in binding buffer, and the suspension is centrifuged. This is repeated appropriately (twice) to remove any unbound labeled compound. The precipitate is again suspended in binding buffer. The resulting suspension is transferred into a scintillation vial, and a scintillator is added. Radioactivity is measured using a liquid scintillation counter.

The specific binding of the labeled compound to the GWT1 protein can be confirmed by assessing whether binding of the labeled compound is inhibited by adding a large excess of unlabeled compound (ten times or more), and whether the compound binds negligibly to membrane fractions prepared from fungal cells which do not express the GWT1 protein.

(c) Binding inhibition of a labeled compound by a test sample

A test sample and the labeled compound are added to the prepared membrane fraction, and the mixture is allowed to stand on ice for an appropriate period of time, for example, one to two hours, while the binding reaction to the membrane fraction takes place. Test compounds used in the present invention's screening method include:

a simple naturally-occurring compound, an organic compound, an inorganic compound, a protein, or a peptide, as well as a compound library, an expression product of a genetic library, a cell extract, a cell culture supernatant, a product from fermentative bacteria, an extract of a marine organism, a plant extract, and the like.

The mixture is centrifuged, for example at 15,000 rpm for three minutes to precipitate the membrane fraction. The precipitate is resuspended in binding buffer and the suspension is centrifuged. This is repeated appropriately (twice) to remove any unbound labeled compound. The precipitate is suspended in the binding buffer. The suspension is transferred into a scintillation vial, and scintillator is added thereto. The radioactivity is measured using a liquid scintillation counter.

When the binding of the labeled compound to the membrane fraction is inhibited in the presence of a test sample, the test sample is judged to have the activity of binding to the malaria parasite GWT1 protein.

[2] The GlcN-PI acyltransferase assay system for screening compounds that inhibit the GlcN-PI acyltransferase activity of the malaria parasite GWT1 protein

The transfer of an acyl group to GPI can be detected by the method reported by Costello L.C and Orlean P., J. Biol. Chem. (1992) 267:8599-8603; or Franzot S.P and Doering T.L., Biochem. J. (1999) 340:25-32. A specific example of the method is described below. The following experimental conditions are preferably optimized for each malaria parasite GWT1 protein to be used.

The malaria parasite GWT1 protein is prepared according to the procedure described in Section 1. A membrane fraction comprising the malaria parasite GWT1 protein is added to a buffer which comprises an appropriate metal ion (Mg^{2+} , Mn^{2+}), ATP, Coenzyme A, and preferably an inhibitor that prevents the consumption of UDP-GlcNAc in other reactions, for example, nikkomycin Z as an inhibitor of chitin synthesis, or tunicamycin as an inhibitor of asparagine-linked glycosylation. A test sample is then added to the mixture and the resulting mixture is incubated at an appropriate temperature for an

appropriate period of time (for example, at 24°C for 15 min).

A GlcN-(acyl)PI precursor (for example UDP-GlcNAc, Acyl-Coenzyme A, and preferably UDP-[¹⁴C]GlcNAc) which has been appropriately labeled, and preferably radiolabeled, is added to the mixture. The resulting mixture is incubated for an appropriate period of time (for example, at 24°C for one hour). A mixture of chloroform and methanol (1:2) is added, the resulting mixture is stirred to halt the reaction, and the lipids are extracted. The extracted reaction product is dissolved in an appropriate solvent, preferably butanol. Then, GlcN-(acyl)PI produced in the reaction is separated by a method such as HPLC or thin layer chromatography (TLC), preferably TLC. When TLC is used, the developer can be selected appropriately from, for example, CHCl₃/CH₃OH/H₂O (65:25:4), CHCl₃/CH₃OH/1M NH₄OH (10:10:3), and CHCl₃/pyridine/HCOOH (35:30:7). A preferred developer is CHCl₃/CH₃OH/H₂O (65:25:4). The separated GlcN-(acyl)PI is quantified using a method appropriate for the label used. When labeled with a radioisotope, the separated GlcN-(acyl)PI can be quantified based on its radioactivity.

When the amount of GlcN-(acyl)PI produced is reduced in the presence of a test sample, the test sample is judged to have the activity of inhibiting acyl group transfer by the malaria parasite GWT1 protein.

[3] A GPI-anchored protein detection system which comprises expressing the malaria parasite GWT1 protein in cells and detecting the GPI-anchored protein on the cell surface

The ability of a test sample to inhibit the activity of the malaria parasite GWT1 protein can be determined using a GPI-anchored protein detection system that comprises expressing the GWT1 protein in cells, preferably fungal cells, and then detecting the GPI-anchored protein on the cell surface. The fungi of the present invention are those belonging to Zygomycota, Ascomycota, Basidiomycota, and Deuteromycete, and preferably pathogenic fungi, *Mucor*, *Saccharomyces*, *Candida*, *Cryptococcus*, *Trichosporon*, *Malassezia*, *Aspergillus*, *Trichophyton*, *Microsporum*, *Sporothrix*, *Blastomyces*, *Coccidioides*, *Paracoccidioides*, *Penicillium*, and *Fusarium*, more preferably *C.*

albicans, *C. glabrata*, *C. neoformans*, and *A. fumigatus*, and even more preferably, yeast. Such yeasts include *S. cerevisiae* and *S. pombe*. The method for introducing into the above-described fungal cells an expression vector containing inserted DNA encoding the malaria parasite GWT1 protein is known to those skilled in the art.

When the malaria parasite GWT1 protein is expressed in fungal cells, the amount of GPI-anchored protein transported to the fungal cell wall can be determined by the following methods: (1) by using a reporter enzyme; (2) by using an antibody that reacts with the surface glycoprotein of fungal cell walls; (3) by using the protein's ability to adhere to animal cells; or (4) by observing fungal cells under a light microscope or electron microscope.

The methods of (1) to (4) have been disclosed in WO 02/04626, which is described specifically in Examples of this invention. The methods (1) to (4), and preferably a combination of these methods (1) to (4), can determine whether a test sample inhibits the transport of the GPI-anchored protein onto the cell wall, or the expression of the GPI-anchored protein on the fungal cell surface.

Hereinafter, the methods of (1) to (4) will be described.

(1) A method using a reporter enzyme

The process that transports GPI-anchored proteins to the cell wall can be quantified using a tracer experiment such as one where a GPI-anchored protein is labeled with a radioactive isotope, the fungal cell wall fraction is obtained, and immunoprecipitated using an antibody against the GPI-anchored protein. Alternatively, quantification can be more readily performed as follows: the C-terminal sequence, which is considered to function as a transport signal and is commonly observed among GPI-anchored proteins, can be expressed as a fusion protein with an easily measurable enzyme (reporter enzyme), the fungal cell wall fraction can be obtained,; and a reporter system that measures the enzyme activity of each fraction can be used (Van Berkel MAA et al., FEBS Letters, 349: 135-138, 1994). Hereinafter, a method which uses a reporter enzyme will be described, but in the present invention such methods are not to be construed as being limited thereto.

First, the reporter gene is constructed and introduced into

fungi. The reporter gene is constructed by linking a promoter sequence that functions in fungi with DNAs that respectively encode a signal sequence, a reporter enzyme, and a GPI-anchored protein C-terminal sequence in such a way that the reading frames match. Examples of the promoter sequence are GAL10 and ENO1. Examples of the signal sequence include α -factor, invertase, and lysozyme. Examples of reporter enzymes are β -lactamase, lysozyme, alkaline phosphatase, and β -galactosidase. Green Fluorescence Protein (GFP), which has no enzyme activity but can be easily detected, can also be used. GPI-anchored protein C-terminal sequences include the α -agglutinin C-terminal sequence, the CWP2 C-terminal sequence, and so on. Furthermore, it is preferable to insert an appropriate selection marker, such as LEU2 and URA3, into the vector comprising the constructed reporter gene.

The constructed reporter gene is inserted into fungi using an appropriate method, such as the lithium acetate method (Gietz D et al., Nucl. Acids Res. 20: 1425, 1992). The fungi are then cultured, as necessary, using a method that suits the selection marker (e.g. using Leu⁻ medium for LEU2 and Ura⁻ medium for URA3), and then fungi into which the DNA has been introduced are selected.

The effect of a test sample on the transport of GPI-anchored proteins to the cell wall is examined by the following method:

The reporter gene-introduced fungi are cultured under appropriate conditions, for example at 30°C for 48 hours, in the presence of a test sample. After culturing, the culture supernatant is centrifuged, and the reporter enzyme activity of the culture supernatant fraction is measured. The resulting cell fraction is washed, the cell wall components are separated using an appropriate method, such as degrading the cell wall glucan with glucanase, and then the reporter enzyme activity of the cell wall fraction and cytoplasmic fraction is measured. The assay can be simply carried out by using centrifugation to determine the amount of reporter enzyme in the cell fraction, then without washing the cells, using proportional calculations to determine the amount of reporter enzyme derived from the culture supernatant fraction that remains in the cell fraction, and subtracting this from the amount of reporter enzyme

of the cell fraction.

If the test sample exhibits the activity of increasing reporter enzyme activity within the culture supernatant fraction (activity per cell), or the activity of decreasing the reporter enzyme activity in the cell wall fraction (activity per cell), the test sample is judged to have influenced the transport process of GPI-anchored proteins to the cell wall.

(2) A method using an antibody that reacts with the surface glycoprotein of fungal cell walls

A test sample's ability to influence the expression of a GPI-anchored protein at the fungal surface layer can be determined by quantification using an antibody that reacts with that GPI-anchored protein in the fungal cell wall.

Antibodies can be obtained by predicting the antigenic determinant using the amino acid sequence of, for example, a GPI-anchored protein such as α -agglutinin, Cwp2p, or Als1p (Chen MH et al., J. Biol. Chem., 270:26168-26177, 1995; Van Der Vaat JM et al., J. Bacteriol., 177:3104-3110, 1995; Hoyer LL et al., Mol. Microbiol., 15:39-54, 1995), and then synthesizing the peptide of that region, binding it to an antigenic substance such as a carrier protein, and then immunizing a rabbit or such to obtain polyclonal antibodies, or a mouse or such to obtain a monoclonal antibody. A rabbit polyclonal antibody against the Als1p peptide is preferable.

In an alternative method, a monoclonal antibody against a GPI-anchored protein may be obtained by immunizing mice and such with fungi, preferably fungi which overexpress a GPI-anchored protein such as α -agglutinin, Cwp2p, and Als1p, (in some cases by immunizing further with a partially purified GPI-anchored protein), and then using ELISA, Western blot analysis, and so on to select resultant clones based on the antibody that they produce.

The following method can be used to determine the influence of a test sample on the process that transports a GPI-anchored protein to the cell wall, and on the amount of protein derived from that GPI-anchored protein in the cell wall.

Fungi are cultured in the presence of a test sample under appropriate conditions such as 30°C for 48 hours. The cultured fungi

are collected by centrifugation and the cells are disrupted, preferably using glass beads. The washed, disrupted cells are preferably subjected to centrifugal extraction with SDS, and then the precipitate is washed. After extraction, the disrupted cells are treated with an enzyme that degrades glucan, preferably glucanase, and the centrifuged supernatant thereof is the GPI-anchored protein sample.

The anti-Als1p peptide antibody is coated onto a 96-well plate by overnight incubation at 4°C. The plate is washed with a washing solution, preferably PBS comprising 0.05% Tween 20 (PBST), and blocking is carried out using a reagent that blocks the non-specific adsorption sites of the 96-well plate, preferably a protein such as BSA or gelatin, more preferably BlockAce (Dainippon Pharmaceutical Co., Ltd.). The plate is again washed with a washing solution, preferably PBST, and an appropriately diluted GPI-anchored protein sample is added. The reaction is then carried out for an appropriate time such as two hours at room temperature. After washing with a washing solution, preferably with PBST, an antibody against the enzyme-labeled *C. albicans*, preferably HRP-labeled anti-*Candida* antibody, is reacted for an appropriate time such as two hours at room temperature. The labeling method may be enzyme labeling or radioactive isotope labeling. After washing with a washing solution, preferably PBST, the amount of Als1p in the GPI-anchored protein sample is calculated by a method appropriate to the type of label, i.e. for an enzyme label, by adding a substrate solution and then, upon stopping the reaction, measuring absorbance at 490 nm.

(3) A method using the ability to adhere to animal cells

The test sample's influence on the expression of a GPI-anchored protein on the fungal surface can be determined by measuring the activity of that GPI-anchored protein in the fungal cell wall, and preferably by measuring the ability of fungi to adhere to animal cells and the like. In addition to the activity of Als1p, Hwplp and such in adhesion to animal cells, GPI-anchored protein activity includes that of α -agglutinin in mating, of Flo1p in yeast aggregation, and so on. Hereinafter, a method using the ability of fungi to adhere to animal cells will be described in detail, but the

present invention is not to be construed as being limited thereto.

A fungus with the ability to adhere to cells is used, and this fungus is preferably *C. albicans*. For mammalian cells, cells that adhere to the fungus, preferably intestinal epithelial cells, are used. The mammalian cells are cultured and fixed using an appropriate method, such as ethanol fixation. The test sample and the fungi are incubated for an appropriate time such as 48 hours at 30°C, then inoculated and cultured for a set time, for example, one hour at 30°C. The culture supernatant is then removed, and the cells are washed with a buffer and overlaid with agar media such as Sabouraud Dextrose Agar Medium (Becton Dickinson Company, Ltd.). After culturing at 30°C overnight, the number of colonies is counted, and the adhesion rate is calculated.

If, when compared to fungi not treated with the compound, a test sample is observed to have the activity of decreasing the number of colonies formed by cell adhesion, that test sample is judged to have influenced the process that transports GPI-anchored proteins to the cell wall.

(4) A method for observing fungi using an electron microscope or an optical microscope

The influence of a test sample on the expression of the GPI-anchored protein in the fungal surface can be determined by observing the structure of the fungal cell wall using an electron microscope.

In the presence of a test sample, a fungus such as *C. albicans* is cultured for a certain period of time, for example, 48 hours at 30°C, and its ultrafine morphological structure is observed using a transmission electron microscope. Herein, observation using a transmission electron microscope can be carried out, for example by the method according to the Electron Microscope Chart Manual (Medical Publishing Center). The flocculent fibrous structure of the outermost layer of a fungal cell has a high electron density and is observable by transmission electron microscope. This structure is not influenced by other existing antifungal agents and is considered to be a surface glycoprotein layer, including GPI-anchored proteins as its constituents. When this structure disappears, leaving only

a slight layer with a high electron density, the test sample is judged to have influenced the process that transports GPI-anchored proteins to the cell wall, compared to untreated cells.

When observation under both a transmission electron microscope and an optical microscope reveals greatly swollen fungal cells and inhibited budding (division), the test sample is judged to have an influence on the cell wall.

The present invention also provides a method for treating malaria, which comprises the step of administering a compound that inhibits the activity of a GWT1 protein in a malaria parasite. Such a compound includes the compounds described in WO 02/04626 (for example, the compounds described herein in (1)-(5)).

The nucleotide sequence for the natural PfGWT1 protein is characterized by an exceedingly high AT content (80.41%), and thus codon usage is biased. In addition, the gene contains sequence stretches comprising six or more consecutive A residues at 23 separate positions, and these sequence stretches may serve as pseudo-poly(A) sites, thus producing truncated proteins. Because of the features described above, the gene was only expressed poorly in yeast, and very difficult to amplify using PCR or to replicate in *E. coli*. It was also difficult to determine the nucleotide sequence. However, the present inventors succeeded in expressing the PfGWT1 protein with a high efficiency by using a degenerate mutant of the DNA (SEQ ID NO: 5), with a lower AT content than the DNA encoding the PfGWT1 protein. The inventors also revealed that the introduction of the degenerate mutant DNA can rescue the phenotype of GWT1-deficient yeast. This finding suggests that the GPI synthase of a malaria parasite is interchangeable with that of a fungus such as yeast.

The AT content of the gene encoding the malaria parasite GPI synthase is, for example, 79.35% for GPI8 and 77.89% for the GPI13 of *P. falciparum*. These AT contents are as high as that of PfGWT1. It is predicted that most *P. falciparum* genes are hardly expressed in other species, because the average AT content over the translated regions of the *P. falciparum* genome is 76.3%. The present inventors succeeded in expressing a degenerate mutant of the DNA with a lower AT content than that of the DNA encoding the PfGWT1 protein, in yeast.

Hence, the malaria parasite GPI synthase can be expressed in a host other than malaria parasites by using such a degenerate DNA mutant. Furthermore, GPI-deficient yeast and GWT1-deficient yeast are known to exhibit similar phenotypes, including the characteristic of lethality and such. Thus, the phenotype of the GPI synthase gene-deficient fungus can be rescued by using the degenerate mutant DNA described above.

The phenotype of the GPI synthase gene-deficient fungus into which the degenerate mutant DNA described above has been introduced depends on the activity of the malaria parasite GPI synthase. Accordingly, compounds that inhibit the activity of the malaria parasite GPI synthase can be selected by screening using the phenotype of the GPI synthase gene-deficient fungus as an index. Thus, antimalarial drugs targeting the GPI biosynthesis pathway can be selected without actually using the malaria parasites themselves.

The present invention provides a degenerate mutant DNA encoding a protein that has the activity of rescuing the phenotype of a GPI synthase gene-deficient fungus, and which has an AT content lower than that of the original DNA encoding the protein involved in the biosynthesis of GPI. Such a DNA can be used in the screening method of the present invention.

As used herein, the term "AT content" refers to the content of adenine and thymine in the entire nucleotide sequence of the coding region of the GPI synthase gene. The AT content in the degenerate mutant DNA of the present invention preferably ranges from 50% to 70%, more preferably from 53% to 65%, and still more preferably from 55% to 62%.

The phenotype of the GPI synthase gene-deficient fungus includes temperature sensitivity (preferably, sensitivity to high temperatures) and lethality.

The proteins of the present invention involved in the biosynthesis of GPI in malaria parasites include GWT1, GPI1, GPI8, GPI3/PIG-A, GPI10/PIG-B, YJR013W/PIG-M, GPI13/PIG-O, GAA1/GAA-1, DPM1, GPI2, GPI15, YDR437W, GPI12, MCD4, GPI11, GPI7, GPI17, GPI16, CDC91, DPM2, DPM3, and SL15. Of the proteins indicated above, GPI1 and GPI8 have been found to be present in malaria parasites, and

GPI3/PIG-A, GPI10/PIG-B, YJR013W/PIG-M, GPI13/PIG-O, GAA1/GAA-1, and DPM1 have been suggested to be present in malaria parasites (Delorenzi et al., Infect. Immun. 70: 4510-4522, 2002). The nucleotide sequences of GWT1, GPI1, GPI8, GPI3/PIG-A, GPI10/PIG-B, YJR013W/PIG-M, GPI13/PIG-O, GAA1/GAA-1, and DPM1 of *P. falciparum* are shown in SEQ ID NO: 1 and the even sequence identification numbers in SEQ ID NOs: 6-21, respectively. Each corresponding amino acid sequence is shown in SEQ ID NO: 2 and the odd sequence identification numbers in SEQ ID NOs: 6-21. In addition, the nucleotide sequence of *P. vivax* GWT1 is shown in SEQ ID NO: 3, and the corresponding amino acid sequence is shown in SEQ ID NO: 4. Using a method known to those skilled in the art, for example, a method using hybridization or PCR, GWT1, GPI1, GPI8, GPI3/PIG-A, GPI10/PIG-B, YJR013W/PIG-M, GPI13/PIG-O, GAA1/GAA-1, or DPM1 of other malaria parasites can be cloned using DNA comprising any one of the nucleotide sequences shown in SEQ ID NO: 1 and 3, and the even-numbered SEQ ID NOs: 6-21.

Furthermore, GPI synthase genes other than GWT1, GPI1, GPI8, GPI3/PIG-A, GPI10/PIG-B, YJR013W/PIG-M, GPI13/PIG-O, GAA1/GAA-1, and DPM1 of malaria parasites can be cloned by using yeast or human GPI synthase genes. The nucleotide sequences of GPI2, GPI15, YDR437W, GPI12, MCD4, GPI11, GPI7, GPI17, GPI16, and CDC91 of yeast (*S. cerevisiae*) are shown in the even sequence identification numbers in SEQ ID NOs: 22-41 respectively; and each corresponding amino acid sequence is shown in the odd sequence identification numbers in SEQ ID NOs: 22-41. In addition, the nucleotide sequences of human DPM2, DPM3, and SL15 are shown in the even sequence identification numbers in SEQ ID NOs: 42-47 respectively; and each corresponding amino acid sequence is shown in the odd sequence identification numbers in SEQ ID NOs: 42-47.

The production of a degenerate mutant DNA encoding a protein involved in the biosynthesis of the GPI of malaria parasites, and with a lower AT content than that of the original DNA, consists of two steps: design, and synthesis. In the design step, the amino acid sequence of a protein of interest is first reverse-translated and then possible codons for each amino acid residue are listed. Reverse translation can be achieved by using commercially available gene

analysis software (for example, DNASIS-Pro; Hitachi Software Engineering Co., Ltd). Of the codons listed, those meeting the purpose (for example, codons whose AT content is lower and codons frequently used in the host to be used for gene expression) are selected for each amino acid. The degenerate mutant DNA can be designed by rearranging the amino acid sequence of the protein of interest using these selected codons.

The DNA thus designed can be synthesized by a method known to those skilled in the art. The degenerate mutant DNA of the present invention can be synthesized based on the designed nucleotide sequence by, for example, using a commercially available DNA synthesizer.

The present invention also provides vectors in which the above-described degenerate mutant DNA has been inserted, and transformants (preferably GPI synthase gene-deficient fungi) that retain the DNA or the vector in an expressible state. The vector and the host can be those described above.

As used herein, the expression "deficient in the GPI synthase gene" means that the functional product of the gene is not expressed, or that the expression level is decreased. The GPI synthase gene-deficient fungus of the present invention can be prepared by disrupting the GPI gene. The disruption can be achieved by inserting DNA unrelated to the gene, for example a selection marker, based on homologous recombination technology, and the like. More specifically, such a mutant fungus can be prepared by introducing into yeast a selection marker cassette which comprises the *his5* gene or the kanamycin resistance gene of *S. pombe* (Longtine *et al.*, Yeast, 14: 953-961, 1998) amplified with primers, each of which comprises a nucleotide sequence homologous to a portion of the gene (ranging from 50 to 70 nucleotides).

The GPI synthase gene-deficient fungus of the present invention includes, for example, the GWT1 temperature-sensitive mutant strain *gwt1-20*, GPI7 disruptant strain, GPI8 mutant strain *gpi8-1*, and GPI10 temperature-sensitive mutant strain *per13-1*.

A GPI synthase gene-deficient fungus which has been transformed with the degenerate mutant DNA of the present invention can be prepared by introducing into a fungus a vector into which the degenerate mutant

DNA has been inserted. pRS316, YEp351, or such can be used as the vector for *S. cerevisiae*, and pCL, pALSK, or such can be used as the vector for *S. pombe*.

The present invention also provides a method of screening for antimalarial drugs, which comprises using GPI synthase gene-deficient fungi described above.

In such a method, the first step comprises contacting a test sample with a GPI synthase gene-deficient fungus that has been transformed with degenerate mutant DNA with a lower AT content than the DNA encoding a protein involved in the biosynthesis of GPI of malaria parasites. The "contact" can be achieved by adding a test sample to the culture of the above-mentioned fungus. When the test sample is a protein, a vector comprising DNA encoding the protein can be introduced into the above-mentioned fungus.

In the method of the present invention, the next step comprises measuring the degree of growth of the above-mentioned fungus. More specifically, the fungus is inoculated under typical culture conditions, specifically, the fungus is inoculated onto a liquid culture medium such as Yeast extract-polypeptone-dextrose medium (YPD medium) or onto an agar plate, and then incubated at 25 to 37°C for 4 to 72 hours. Thus GPI synthase gene-deficient fungus transformed with the degenerate mutant DNA of the present invention can be assessed for growth. The degree of growth can also be determined using the turbidity of the culture liquid, the number of colonies, or the size or color of the spots formed on the agar plate as an index. In the method of the present invention, the next step comprises selecting compounds that inhibit the growth of the above-mentioned fungus.

In an alternative method, the first step comprises contacting a test sample with a GPI synthase gene-deficient fungus in which the above-described degenerate mutant DNA has been introduced. The next step comprises determining the amount of GPI-anchored protein transported onto the yeast cell wall. The detection method includes: (1) methods using a reporter enzyme; (2) methods using an antibody that reacts with a surface glycoprotein on the fungal cell wall; (3) methods using the ability to adhere to animal cells; and (4) methods using a light microscope or an electron microscope to observe the

fungi. In the method of the present invention, the next step comprises selecting a sample that decreases the amount of GPI-anchored protein transported to the cell wall.

The present invention provides a method of screening for antimalarial drugs using a protein involved in the biosynthesis of GPI, which is prepared using a degenerate mutant DNA of the present invention. Such methods include, for example, a binding assay system where screening is carried out to select compounds that bind to a protein involved in GPI biosynthesis in competition with a labeled compound bound to the protein. Specifically, a degenerate mutant DNA of the present invention is introduced into the GPI synthase gene-deficient fungus, the protein encoded by the DNA is expressed in the fungus, and the expressed protein is prepared. The prepared protein is then contacted with a test sample and with a labeled compound that can bind to the protein. In the next step, the labeled compound bound to the protein is detected, and test samples that decrease the amount of labeled compound bound to the protein are selected.

The present invention also provides an assay system for GlcN-PI acyltransferase. Such a system comprises using a GWT1 protein which is prepared using a DNA encoding a protein that has the activity of complementing the phenotype of GWT1-deficient yeast, which the DNA is a degenerate mutant of a DNA encoding a malaria parasite GWT1 protein that has a lower AT content than the original DNA. Specifically, the degenerate mutant DNA is introduced into GWT1-deficient fungus, the protein encoded by the degenerate mutant DNA is expressed in the fungus, and the expressed protein is prepared. This protein is then contacted with a test sample, GlcN-(acyl)PI is detected, and a test sample that decreases the amount of GlcN-(acyl)PI is selected.

Any patents, patent applications, and publications cited herein are incorporated by reference in their entireties.

Brief Description of the Drawings

Fig. 1 depicts photographs showing the results of tetrad analysis. The gwt1-disrupted strain became viable after the

introduction of the opfGWT1-overexpressing plasmid. The four spores derived from a single diploid cell were spotted vertically.

If one copy of the GWT1 gene was disrupted, only half of the spores grew. Thus, the ratio of [colony-forming spots]: [spots exhibiting no growth] is 2:2 in such cases. In the columns marked with an arrow, the lethal phenotype of the gwt1 disruptant was complemented by the introduced opfGWT1, and hence all four spots grew, each forming a colony.

Fig. 2 depicts a diagram showing the inhibitory activity of a compound with respect to the growth of yeast expressing the opfGWT1 gene. Either the yeast GWT1 gene or opfGWT1 gene was expressed in GWT1 gene-disrupted yeast.

A compound having the activity of inhibiting the GWT1-dependent growth of yeast also showed inhibitory activity with respect to the opfGWT1-dependent growth of yeast in which opfGWT1 was expressed.

Fig 3 depicts a diagram showing antimalarial activity. Human red blood cells were infected with *P. falciparum*. A GWT1-inhibiting compound was added to these red blood cells, and inhibition of malaria parasite infection was determined.

All five compounds exhibiting antifungal activity also inhibited the malaria parasite infection of red blood cells.

Best Mode for Carrying out the Invention

Herein below, the present invention will be specifically described using Examples, but it is not to be construed as being limited thereto.

[Example 1] *P. falciparum* GWT1 (PfGWT1)

(1) The nucleotide sequence of *P. falciparum* GWT1 (PfGWT1) (SEQ ID NO: 1) has been disclosed in the database of the *P. falciparum* genome (PlasmoDB database, <http://plasmodb.org/>). The PfGWT1 gene was cloned by PCR using genomic DNA purified from *P. falciparum* (the 3D7 strain) as a template. The 5' half and 3' half of the PfGWT1 gene were prepared separately, and the two halves were assembled at an XbaI (TCTAGA) restriction enzyme site. Thus, the full-length PfGWT1 gene was prepared. In addition, restriction enzymes sites outside the coding region were included, thus allowing insertion into an

expression vector.

(2) The 5' half of the PfGWT1 gene was amplified by PCR using *P. falciparum* genomic DNA as a template and the primers pf152F (SEQ ID NO: 48) and pf136R (SEQ ID NO: 49). The 3' half was amplified by the same procedure described above, using the primers pf137F (SEQ ID NO: 50) and pf151R (SEQ ID NO: 51). The DNA fragments amplified were subcloned into the pT7-Blue vector (Novagen), and the nucleotide sequences of the inserts were sequenced to confirm homology to SEQ ID NO: 1. Clones containing the 5' half of the PfGWT1 gene were named PF15-5 clones. Clones containing the 3' half were named PF20-9 clones.

(3) Using PCR, cleavage sites for restriction enzymes were added outside the coding region to enable the PfGWT1 gene to be inserted into an expression vector. An EcoRI cleavage site was added to the 5' half by PCR using PF15-5 as a template and the primers pf154FE (SEQ ID NO: 52) and pf157R (SEQ ID NO: 53). The amplified DNA fragment was subcloned into the pT7-Blue vector (Novagen) to prepare the clone pT7-plasmN2. Likewise, the 3' half was amplified by PCR using PF20-9 as a template and the primers pf168BK (SEQ ID NO: 54) and pf155RK (SEQ ID NO: 55). The amplified DNA fragments were subcloned to prepare pT7-plasmBK5 clones.

(4) The full-length PfGWT1 gene was prepared by the procedure described below. The yeast expression vector YEp352GAPII was digested with the restriction enzymes EcoRI and KpnI. The EcoRI-XbaI fragment (about 1500 bp) derived from pT7-plasmN2, and the XbaI-KpnI fragment (about 1100 bp) derived from pT7-plasmBK5, were inserted into the vector at a cleaved site. The expression vector YEp352GAPII-PfGWT1 containing the full-length PfGWT1 was then constructed.

[pf152F] ATGACAATGTGGGGAAGTCAACGGg (SEQ ID NO: 48)

[pf136R] TGTGTGGTTACCGTTCTTTGAATACATAGA (SEQ ID NO: 49)

[pf137F] ATAGAAAATGATTTATGGTACAGCTCAAA (SEQ ID NO: 50)

[pf151R] AGACCAAATTAATTATGCCTTTACATGTAC (SEQ ID NO: 51)

[pf154FE] agaattcaccATGAGCAACATGAATATACTTGCGTATCTT (SEQ ID NO:

52)

[pf157R] GAAATCCAATGTATTCCATATTCATTAT (SEQ ID NO: 53)

[pf168BK] AAGATCTAATACATTAAACATTTTAGATTAATGAATATGTG (SEQ ID NO: 54)

[pf155RK] aggtaccGTACACTCCACTCTATGATGATCATTC (SEQ ID NO: 55)

[Example 2] A fully synthetic PfGWT1 gene

The adenine and thymine (AT) proportion is exceedingly high (80% or higher) in *P. falciparum* DNA, and thus routine biological techniques (PCR, *E. coli*-based gene engineering, expression systems for recombinant proteins, and so on) are often unavailable (Sato and Horii; Protein, Nucleic acid, and Enzyme Vol. 48, 149-155, 2003). Likewise, the AT content of PfGWT1 DNA was 80.41% including many consecutive A or T stretches. Thus, the gene was predicted to be difficult to replicate and express as a protein in yeast. Indeed, when native PfGWT1 ligated with a yeast overexpression vector was introduced into GWT1 disrupted yeast, the PfGWT1 did not rescue the lethal phenotype of the GWT1 disruptant at all. To reduce AT content, codons were replaced with synonymous codons without changing the original amino acid sequence.

The codon substitution was carried out based on the nucleotide sequence of *P. falciparum* GWT1 (SEQ ID NO: 1) disclosed in the *P. falciparum* genome database (PlasmoDB database, <http://plasmodb.org/>). The resulting nucleotide sequence was named "optimized PfGWT1 (opfGWT1)" (SEQ ID NO: 5).

The sequence described above was designed to include additional sequences outside the coding region; namely an EcoRI cleavage site sequence (GAATTC, at the 5' end), Kozak's sequence (ACC, at the 5' end), and a KpnI cleavage site sequence (GGTACC, at the 3' end). The synthesis of the resulting sequence was consigned to Blue Heron Inc. in the U.S.A. These additional restriction enzyme sites were used to ligate the fully synthetic opfGWT1 into the YEp352GAPII vector to construct an overexpression plasmid for opfGWT1. The construct was introduced into diploid cells (WDG2) in which only a single copy of the GWT1 gene had been disrupted. The resulting transformants were cultured on plates containing a sporulation medium to form spores for tetrad analysis.

The AT content of the newly designed codon-modified opfGWT1 was

reduced to 61.55%. The results of tetrad analysis are shown in Fig. 1. The *gwt1*-disrupted strain became viable after introduction of the *opfGWT1* overexpression plasmid. The findings described above indicate that the *PfGWT1* gene can be expressed in yeast cells when its AT content is reduced by codon modification.

[Example 3] An assay for antimalarial activity using *opfGWT1*-expressing yeast

A screening system for compounds having antimalarial activity was constructed using *opfGWT1*-expressing yeast.

An expression cassette was constructed by inserting the *S. cerevisiae* *GWT1* terminator, and the *S. cerevisiae* GAPDH promoter and multi-cloning site into the *SacI*-*KpnI* site of the single-copy vector pRS316. *S. cerevisiae* *GWT1* and *opfGWT1* were inserted into the multi-cloning site to prepare pGAP-*ScGWT1* and pGAP-*opfGWT1* plasmids, respectively. These plasmids were introduced into the *GWT1* disruptant. Serial two-fold dilutions of compound (1) were prepared using YPAD to make the highest final concentration 50 µg/ml. A 50 µl aliquot of the diluted compound was added to each well of a 96-well plate. Overnight cultures of yeast cells comprising each plasmid were diluted 1000-fold and then a 50 µl aliquot of the dilution was added to each well. The plates were incubated at 30°C for two days, and then culture turbidity was determined at 660 nm (Fig. 2 and Table 1).

[Table 1]

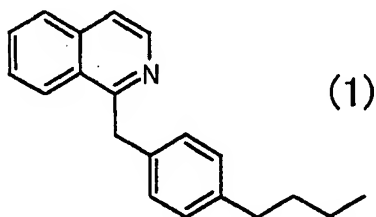
	0	6.25	12.5	25	50
pGAP- <i>ScGWT1</i>	0.7560	0.7370	0.6670	0.1140	0.0420
pGAP- <i>opfGWT1</i>	0.7150	0.6990	0.6910	0.3630	0.0530

Although the *GWT1* disruptant was nonviable, the strain became viable after introduction of each plasmid (as shown at 0 µg/ml of compound concentration). The growth of *ScGWT1*-expressing yeast was inhibited by compound (1), a *GWT1*-specific inhibitor. The use of the

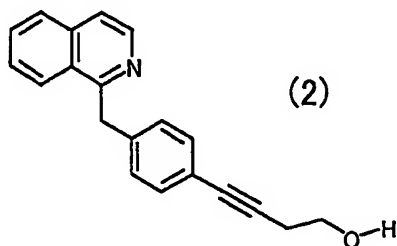
compound at 25 µg/ml resulted in about 85% inhibition of growth. When the compound was used at 50 µg/ml, the yeast was completely nonviable. The growth of opfGWT1-expressing yeast was also inhibited by compound (1). The use of the compound at 25 µg/ml resulted in about 50% inhibition of growth. When the compound was used at 50 µg/ml, the yeast was completely nonviable. Since growth of opfGWT1-expressing yeast depends on the activity of the introduced opfGWT1, growth inhibition can be attributed to the inhibition of the opfGWT1 function by compound (1). These findings suggest that compounds with *P. falciparum* GWT1-specific inhibitory activity GWT1 can be identified by screening compounds using this assay system.

[Example 4] Antimalarial activity

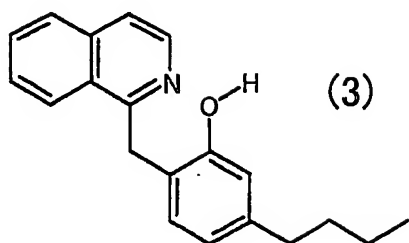
Representative compounds (1) to (5), that inhibit yeast GWT1, were assayed for antimalarial activity using a red blood cell culture system.



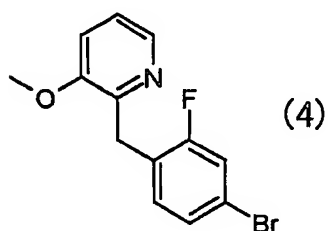
compound (1): 1-(4-butyl benzyl) isoquinoline



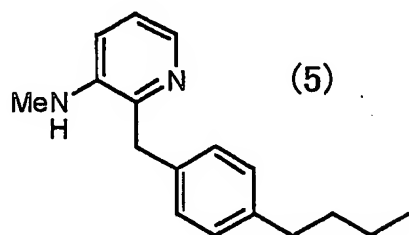
compound (2): 4-[4-(1-isoquinolyl methyl) phenyl]-3-butyne-1-ol



compound (3): 5-butyl-2-(1-isoquinolyl methyl) phenol



compound (4): 2-(4-bromo-2-fluorobenzyl)-3-methoxypyridine



compound (5): N-[2-(4-butyl benzyl)-3-pyridyl]-N-methylamine

Specifically, a test compound was dissolved in 100% DMSO, diluted with a medium, and an 80 μ l aliquot of the dilution was added to each well of a 96-well culture plate. *P. falciparum* FCR3 strain was pre-cultured in RPMI1640 medium containing 10% human serum at 37°C, and then 20 μ l of the cultured cells (containing 10% red blood cells) was added to each well. At this time, 0.47% of red blood cells were infected. After culturing under 5% O₂, 5% CO₂, and 90% N₂ at 37°C for 48 hours, the malaria parasites were stained using Giemsa staining. The number of protozoan-infected red blood cells was determined in order to estimate infection rate (Fig 3). As a result, compound (3) was revealed to have strong antimalarial activity. The other four compounds also showed antimalarial activity. Compound (4) exhibited the lowest activity. Therefore, compounds inhibiting yeast GWT1 include compounds which have the activity of inhibiting *P. falciparum*.

GWT1, suggesting that antimalarial drugs can be synthesized based on such compounds.

Industrial Applicability

The present invention succeeded in producing fungi that express malaria parasite GWT1. Using such fungi, antimalarial drugs targeting the pathway of GPI biosynthesis can be screened without using malaria parasites.

To date, no attempt has been made to express a malaria parasite gene in fungal cells and screen substances which inhibit the function of that gene. The methods of the present invention remove the need to actually using malaria parasites themselves, and thus this method proves the possibility of entirely new screening methods for drug discovery using comparative genomics in the post-genome era.

CLAIMS

1. A DNA according to any one of (a) to (d), which encodes a protein of a malaria parasite having GlcN-PI acyltransferase activity:

(a) a DNA encoding a protein comprising the amino acid sequence of SEQ ID NO: 2 or 4,

(b) a DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3,

(c) a DNA hybridizing to DNA comprising the nucleotide sequence of SEQ ID NO: 1 or 3 under stringent conditions, and

(d) a DNA encoding a protein which comprises the amino acid sequence of SEQ ID NO: 2 or 4, in which one or more amino acids have been added, deleted, substituted, and/or inserted.

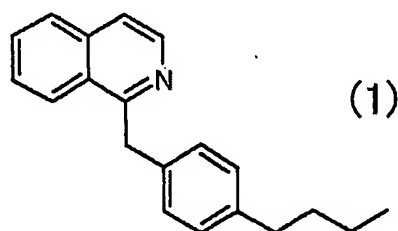
2. A protein encoded by the DNA according to claim 1.

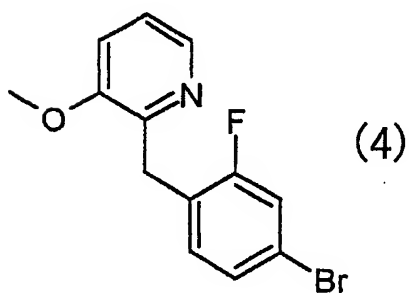
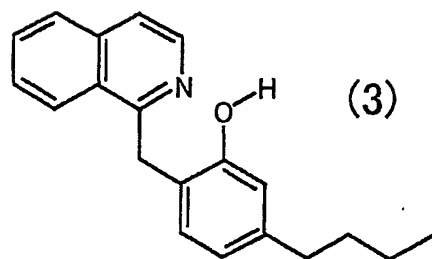
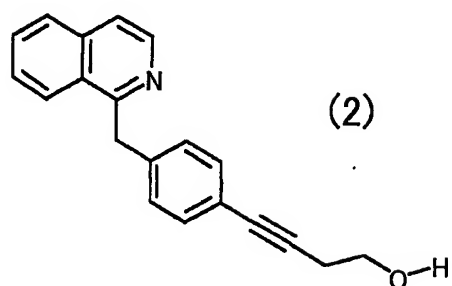
3. A vector into which the DNA according to claim 1 is inserted.

4. A transformant which retains, in an expressible state, the DNA according to claim 1 or the vector according to claim 3.

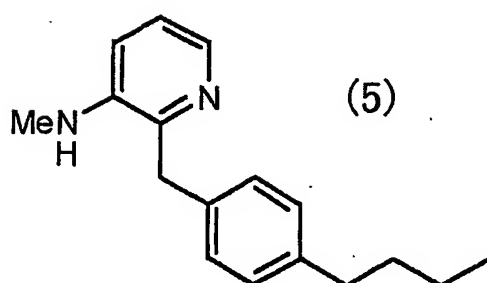
5. An antimalarial drug which comprises as an active ingredient a compound that inhibits the activity of the protein according to claim 2.

6. The antimalarial drug according to claim 5, wherein the compound that inhibits the activity of the protein according to claim 2 is at least one selected from the group consisting of the following compounds (1) to (5):





and,



7. A method of screening for a compound having antimalarial activity, which comprises the steps of:

(1) contacting the protein according to claim 2 with a test sample and a labeled compound that has the activity of binding to the protein,

(2) detecting the labeled compound that binds to the protein,
and,

(3) selecting a test sample that decreases the amount of labeled compound that binds to the protein.

8. The method according to claim 7, wherein the labeled compound that has the activity of binding to the protein is produced by labeling at least one compound selected from the group consisting of the compounds (1) to (5) according to claim 6.

9. A method of screening for a compound having antimalarial activity, which comprises the steps of:

(1) contacting a test sample with the protein according to claim 2,

(2) detecting GlcN-(acyl)PI, and,

(3) selecting a test compound that decreases the level of GlcN-(acyl)PI.

10. A method of screening for a compound having antimalarial activity, which comprises the steps of:

(1) contacting a test sample with a cell overexpressing the protein according to claim 2,

(2) determining the amount of GPI-anchored protein transported to the cell wall in the cell, and,

(3) selecting a test sample that decreases the amount of the GPI-anchored protein transported to the cell wall, as determined in step (2).

11. A method for treating malaria, which comprises administering a compound that inhibits the activity of the protein according to claim 2.

12. The method according to claim 11, wherein the compound that inhibits the activity of the protein according to claim 2 is the compound according to claim 5.

13. A DNA encoding a protein that has the activity of complementing the phenotype of a GPI synthase gene-deficient yeast, which is a degenerate mutant of a DNA encoding a protein involved in GPI biosynthesis in malaria parasites, and that has a lower AT content than the original DNA.

14. A DNA encoding a protein that has the activity of complementing the phenotype of a GPI synthase gene-deficient yeast, which is a degenerate mutant of a DNA encoding a protein involved

in the biosynthesis of GPI in malaria parasites, and that has an AT content which is reduced by 70%.

15. The DNA according to claim 13 or 14, which is selected from the group consisting of:

(a) a DNA encoding a protein that comprises any one of the amino acid sequences of SEQ ID NOs: 2 and 4, and odd sequence identification numbers in SEQ ID NOs: 6-47,

(b) a DNA comprising any one of the nucleotide sequences of SEQ ID NOs: 1 and 3, and even sequence identification numbers in SEQ ID NOs: 6-47,

(c) a DNA hybridizing under stringent conditions to the DNA that comprises any one of the nucleotide sequences of SEQ ID NOs: 1 and 3, and even sequence identification numbers in SEQ ID NOs: 6-47, and

(d) a DNA encoding a protein which comprises any one of the amino acid sequences of SEQ ID NOs: 2 and 4, and odd sequence identification numbers in SEQ ID NOs: 6-47, in which one or more amino acids have been added, deleted, substituted, and/or inserted.

16. A DNA comprising the nucleotide sequence of SEQ ID NO: 5.

17. A vector in which the DNA according to any one of claims 13 to 16 is inserted.

18. A transformant which retains, in an expressible state, the DNA according to any one of claims 13 to 16, or the vector according to claim 17.

19. The transformant according to claim 18, which is a GPI synthase gene-deficient fungus.

20. The transformant according to claim 18, which is a GPI synthase gene-deficient yeast.

21. A method for producing a protein encoded by the DNA according to any one of claims 13 to 16, which comprises the steps of culturing the transformant according to any one of claims 18 to 20, and recovering the expressed protein from the transformant or the culture supernatant.

22. A method of screening for a compound having antimalarial activity, which comprises the steps of:

(1) contacting a test sample with a GPI synthase gene-deficient fungus that expresses the DNA according to any one of claims 13 to

16,

(2) assaying the growth of that fungus, and,

(3) selecting a test compound that inhibits the growth of that fungus.

23. A method of screening for a compound having antimalarial activity, which comprises the steps of:

(1) contacting a test sample with a GPI synthase gene-deficient fungus expressing the DNA according to any one of claims 13 to 16,

(2) determining the amount of a GPI-anchored protein transported to the fungal cell wall, and,

(3) selecting a test sample that decreases the amount of the GPI-anchored protein transported to the cell wall, as determined in step (2).

24. A method of screening for a compound having antimalarial activity, which comprises the steps of:

(1) introducing the DNA according to any one of claims 13 to 16 into a GPI synthase gene-deficient fungus and expressing the protein encoded by the DNA,

(2) preparing the protein expressed in step (1),

(3) contacting the prepared protein with a test sample and a labeled compound that has the activity of binding to the protein,

(4) detecting the labeled compound that binds to the protein, and,

(5) selecting a test sample that decreases the amount of labeled compound that binds to the protein.

25. A method of screening for a compound having antimalarial activity, which comprises the steps of:

(1) introducing into a GWT1-deficient fungus, (i) a DNA encoding a protein that has the activity of complementing the phenotype of a GWT1-deficient yeast, wherein the DNA is a degenerate mutant of a DNA encoding a malaria parasite GWT1 protein that has a lower AT content than the original DNA, or (ii) a vector into which the degenerate mutant of DNA has been inserted, and expressing the protein encoded by the degenerate mutant DNA,

(2) preparing the protein expressed in step (1),

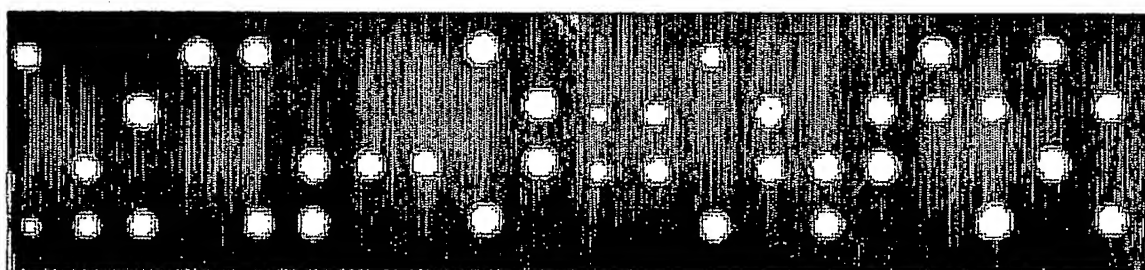
(3) contacting the prepared protein with a test sample,

- (4) detecting GlcN-(acyl)PI, and
- (5) selecting a test compound that decreases the level of GlcN-(acyl)PI.

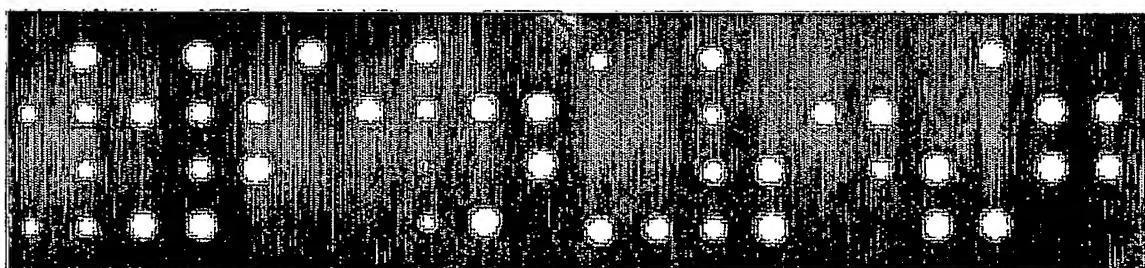
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FIG. 1

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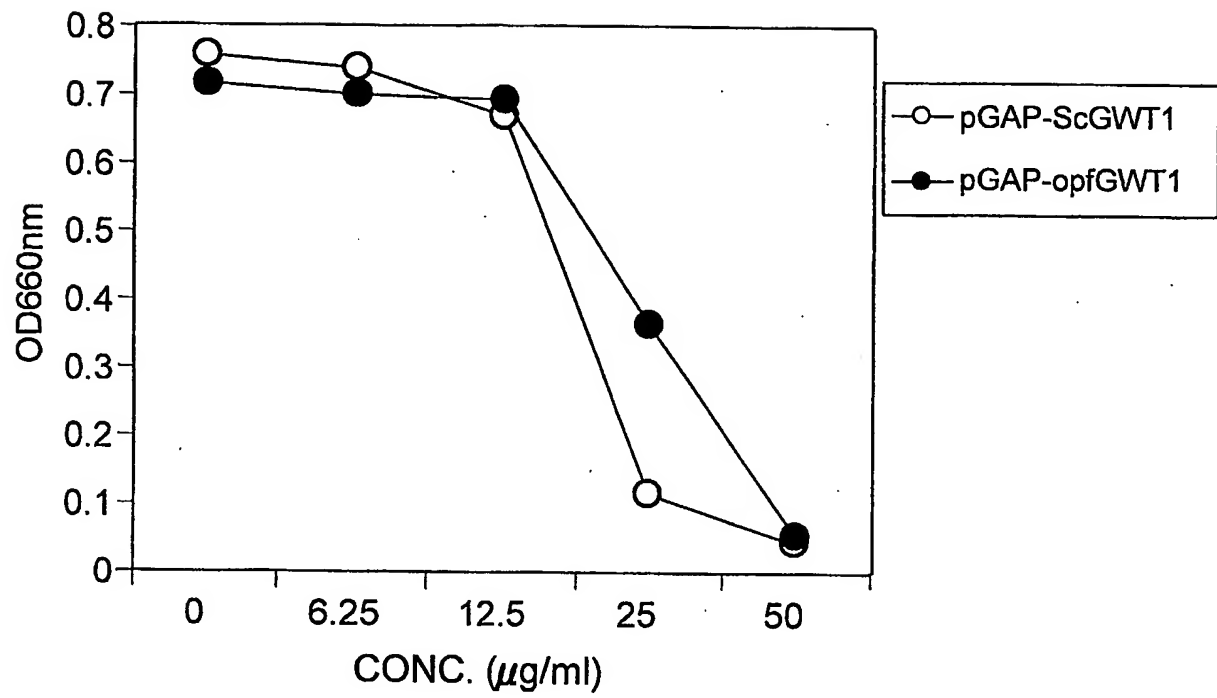


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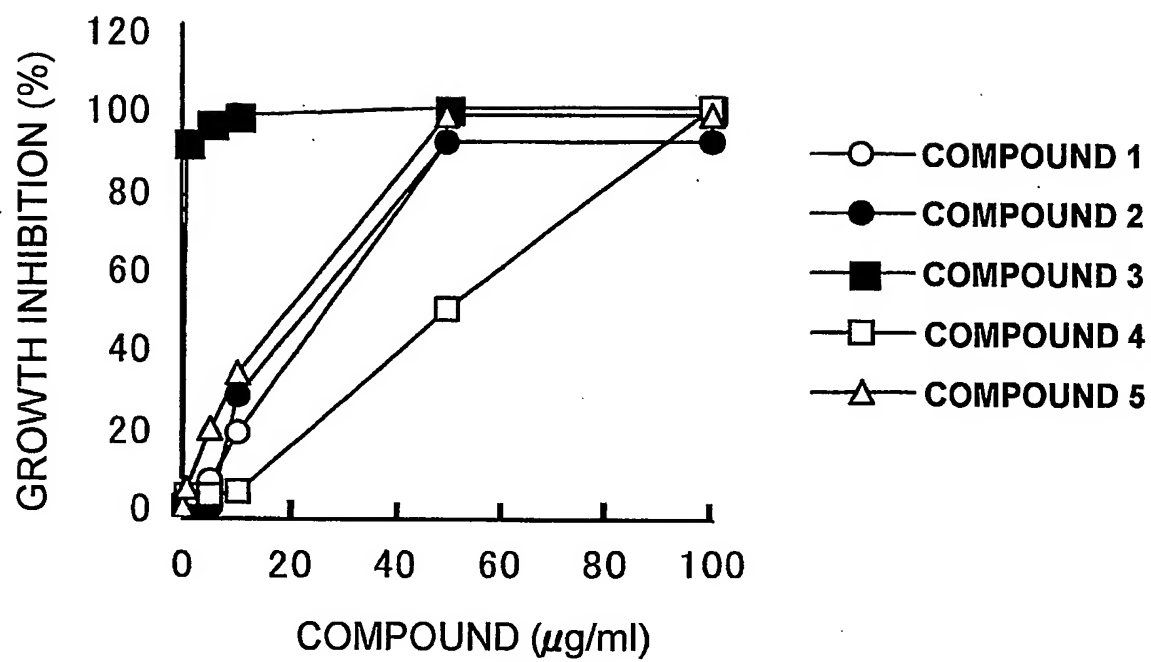
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FIG. 2



3/3

FIG. 3



1 / 1 6 1

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<120> Methods of screening for compounds that inhibit the biosynthesis of
GPI in malaria parasites

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10/161

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1 1 / 1 6 1

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Leu Leu Ile Tyr Lys Asn Glu Lys Thr Lys Ser Ser Ile Lys Phe Ile

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17/161

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Arg Lys Gly Gln Glu Glu Ser Tyr Asn Leu Val Val Gly Ser Phe Gly

85

90

95

aag gaa gcc aaa ggg gag gtc tcc ctc caa aga gtt ctc atc aca aat 336

Lys Glu Ala Lys Gly Glu Val Ser Leu Gln Arg Val Leu Ile Thr Asn

100

105

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gat gcc gtg tac ctg tcg tac cag gat gtg caa aac gaa cgt ggg atc 384

Asp Ala Val Tyr Leu Ser Tyr Gln Asp Val Gln Asn Glu Arg Gly Ile

115

120

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caa gtt aag ata aaa agg ggg gaa ata tct tcc tat tta gac ctc cta 432

Gln Val Lys Ile Lys Arg Gly Glu Ile Ser Ser Tyr Leu Asp Leu Leu

130

135

140

tcg tgg gat tct tgt ttg tat aag ctt aac tca gac gat tat aat tta 480

19 / 161

Ser Trp Asp Ser Cys Leu Tyr Lys Leu Asn Ser Asp Asp Tyr Asn Leu

145 150 155 160

atg aag agc gca tcg gat cat agc aag cca atg gtg gtc agc aca tac 528

Met Lys Ser Ala Ser Asp His Ser Lys Pro Met Val Val Ser Thr Tyr

165 170 175

cac ata tac atg ctg ctg ctg gtg ttt tct ctt tgc act tac gtg gag 576

His Ile Tyr Met Leu Leu Leu Val Phe Ser Leu Cys Thr Tyr Val Glu

180 185 190

aag agc ctc ctg ctt gaa ttc cct gcg ttg aaa aag tgc caa gta ttt 624

Lys Ser Leu Leu Leu Glu Phe Pro Ala Leu Lys Lys Cys Gln Val Phe

195 200 205

cta acc cta tgt ttg gtg tac tgc ccg ata atc agt tac ctt ttt ttt 672

Leu Thr Leu Cys Leu Val Tyr Cys Pro Ile Ile Ser Tyr Leu Phe Phe

210 215 220

ttt tac tcc cat gtg agc cta ctt ggg gtg tta ctc gtc tat gtg ttt 720

Phe Tyr Ser His Val Ser Leu Leu Gly Val Leu Leu Val Tyr Val Phe

225 230 235 240

ttt tgc ggg ctc ttc agg ggc gtc tct tgc aga agg ggg ggg cag cac 768

Phe Cys Gly Leu Phe Arg Gly Val Ser Cys Arg Arg Gly Gly Gln His

245 250 255

20/161

atg ggg gag caa acg ggc caa cac acg ggc gat tgg cac acc atc cgc 816

Met Gly Glu Gln Thr Gly Gln His Thr Gly Asp Trp His Thr Ile Arg

260

265

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ggc aac cca caa ggt gat gat acg caa gag gag aga cgc aag tgt ttg 864

Gly Asn Pro Gln Gly Asp Asp Thr Gln Glu Glu Arg Arg Lys Cys Leu

275

280

285

gtc cat atg agg cta gcc aac ctg tgc atc acc tac ata tgc ata ttc 912

Val His Met Arg Leu Ala Asn Leu Cys Ile Thr Tyr Ile Cys Ile Phe

290

295

300

gct gtg gac ttt tat ttt ttc cca agg caa ttt tcc aag tct ttt ttt 960

Ala Val Asp Phe Tyr Phe Phe Pro Arg Gln Phe Ser Lys Ser Phe Phe

305

310

315

320

ttt ggt aac act ttg atg gat tta ggg gtg ggg ggg tgc atc aca tcg 1008

Phe Gly Asn Thr Leu Met Asp Leu Gly Val Gly Gly Cys Ile Thr Ser

325

330

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agc gcg tat tct cta aac agt aaa aag ctc cat tct gcg aac cgc aag 1056

Ser Ala Tyr Ser Leu Asn Ser Lys Lys Leu His Ser Ala Asn Arg Lys

340

345

350

gga cac cta atc gat tgg aag cat ttc att tta ttt ttc ctt gga ata 1104

21 / 161

Gly His Leu Ile Asp Trp Lys His Phe Ile Leu Phe Phe Leu Gly Ile

355

360

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gct aga tac att gca gtg aag ctt ttc aat tat aat tac agc tta act 1152

Ala Arg Tyr Ile Ala Val Lys Leu Phe Asn Tyr Asn Tyr Ser Leu Thr

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gag tat ggg atg cac tgg aat ttt ttt ctt act ctc ttt ttt act ctc 1200

Glu Tyr Gly Met His Trp Asn Phe Phe Leu Thr Leu Phe Phe Thr Leu

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395

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cta act tgt aac gcc cta ctc tgc ttg ata aga ggg gtt aaa cgc acc 1248

Leu Thr Cys Asn Ala Leu Leu Cys Leu Ile Arg Gly Val Lys Arg Thr

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ttt cac ctg agc tgc gtc ctc atc tgt ttg tat gaa att ata att tgg 1296

Phe His Leu Ser Cys Val Leu Ile Cys Leu Tyr Glu Ile Ile Ile Trp

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cgc ctg gac att acg agt tat tta gtg gtt gac gag gca gaa cgg agc 1344

Arg Leu Asp Ile Thr Ser Tyr Leu Val Val Asp Glu Ala Glu Arg Ser

435

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ggc ttt ttt tcg cag aac aga gag ggc ctt atg aac gtc atc ggg tcc 1392

Gly Phe Phe Ser Gln Asn Arg Glu Gly Leu Met Asn Val Ile Gly Ser

450

455

460

2 2 / 1 6 1

gtc aat ttg tac ctc ttt tcg ttt tcg cta tgg aat ggc tat gtg ttt	1440
Val Asn Leu Tyr Leu Phe Ser Phe Ser Leu Trp Asn Gly Tyr Val Phe	
465 470 475 480	
ccg gat gag ggg cag cag tgg gag cga gga aag gcg gcg cga aga ccg	1488
Pro Asp Glu Gly Gln Gln Trp Glu Arg Gly Lys Ala Ala Arg Arg Pro	
485 490 495	
gat gag gcg gcg cga acg ccg ggg gag gga cat ggc cag cgc tcc cct	1536
Asp Glu Ala Ala Arg Thr Pro Gly Glu Gly His Gly Gln Arg Ser Pro	
500 505 510	
gtc cgc ctc acc ctg aag ttg ctt gcc ctg tcc ctc ctc ttc cac ctg	1584
Val Arg Leu Thr Leu Lys Leu Leu Ala Leu Ser Leu Leu Phe His Leu	
515 520 525	
ctg cac ctg ctg ttg aat tac tac cga aat tac agt gtg cgc atc ctt	1632
Leu His Leu Leu Leu Asn Tyr Tyr Arg Asn Tyr Ser Val Arg Ile Leu	
530 535 540	
tgc aac gcg aac tac ata tgt gtt gtc tcc tcc gtg agt ctc ttc gcg	1680
Cys Asn Ala Asn Tyr Ile Cys Val Val Ser Ser Val Ser Leu Phe Ala	
545 550 555 560	
gct gcc ctg agc tac ctc gta gag aag gta ctc ctc cgc gag aag acc	1728

2 3 / 1 6 1

Ala Ala Leu Ser Tyr Leu Val Glu Lys Val Leu Leu Arg Glu Lys Thr

565

570

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acc acc atc cca gtt ttg caa caa atg aac cgg cac tcc ctg gca gtg 1776

Thr Thr Ile Pro Val Leu Gln Gln Met Asn Arg His Ser Leu Ala Val

580

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ttc ctc ttt tgc aac gta aca atg ggc act ttc aac ctc ctc ttt cag 1824

Phe Leu Phe Cys Asn Val Thr Met Gly Thr Phe Asn Leu Leu Phe Gln

595

600

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tct ctc ttg ttt ccc cta ttt ttt ggc tgc ctc gtt ttg gcg gcg tac 1872

Ser Leu Leu Phe Pro Leu Phe Phe Ala Cys Leu Val Leu Ala Ala Tyr

610

615

620

tcc tat ggc atg ttg cgc ttc gcc tcc ctg ttg ccc ggc ccc gcg cag 1920

Ser Tyr Gly Met Leu Arg Phe Ala Ser Leu Leu Pro Gly Pro Ala Gln

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ggg gag aag gga gag aag cgg gag aag cag caa taa 1956

Gly Glu Lys Gly Glu Lys Arg Glu Lys Gln Gln

645

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24/161

<212> PRT

<213> Plasmodium vivax

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Lys Ala Ala Ser Gly Glu Thr Phe Thr Tyr Gly Ala Thr Ala Arg Glu

35 40 45

Asn Leu Gly Ser Tyr Ser Pro Ala His Asp Glu Leu Tyr Met Leu Glu

50 55 60

Leu Ala Lys Met Tyr Tyr Lys Ile Val Leu Thr Tyr Lys Lys Asp Val

65 70 75 80

Arg Lys Gly Gln Glu Glu Ser Tyr Asn Leu Val Val Gly Ser Phe Gly

85 90 95

Lys Glu Ala Lys Gly Glu Val Ser Leu Gln Arg Val Leu Ile Thr Asn

100 105 110

Asp Ala Val Tyr Leu Ser Tyr Gln Asp Val Gln Asn Glu Arg Gly Ile

25 / 161

115

120

125

Gln Val Lys Ile Lys Arg Gly Glu Ile Ser Ser Tyr Leu Asp Leu Leu

130

135

140

Ser Trp Asp Ser Cys Leu Tyr Lys Leu Asn Ser Asp Asp Tyr Asn Leu

145

150

155

160

Met Lys Ser Ala Ser Asp His Ser Lys Pro Met Val Val Ser Thr Tyr

165

170

175

His Ile Tyr Met Leu Leu Leu Val Phe Ser Leu Cys Thr Tyr Val Glu

180

185

190

Lys Ser Leu Leu Leu Glu Phe Pro Ala Leu Lys Lys Cys Gln Val Phe

195

200

205

Leu Thr Leu Cys Leu Val Tyr Cys Pro Ile Ile Ser Tyr Leu Phe Phe

210

215

220

Phe Tyr Ser His Val Ser Leu Leu Gly Val Leu Leu Val Tyr Val Phe

225

230

235

240

Phe Cys Gly Leu Phe Arg Gly Val Ser Cys Arg Arg Gly Gly Gln His

245

250

255

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Met Gly Glu Gln Thr Gly Gln His Thr Gly Asp Trp His Thr Ile Arg

260

265

270

Gly Asn Pro Gln Gly Asp Asp Thr Gln Glu Glu Arg Arg Lys Cys Leu

275

280

285

Val His Met Arg Leu Ala Asn Leu Cys Ile Thr Tyr Ile Cys Ile Phe

290

295

300

Ala Val Asp Phe Tyr Phe Phe Pro Arg Gln Phe Ser Lys Ser Phe Phe

305

310

315

320

Phe Gly Asn Thr Leu Met Asp Leu Gly Val Gly Gly Cys Ile Thr Ser

325

330

335

Ser Ala Tyr Ser Leu Asn Ser Lys Lys Leu His Ser Ala Asn Arg Lys

340

345

350

Gly His Leu Ile Asp Trp Lys His Phe Ile Leu Phe Phe Leu Gly Ile

355

360

365

Ala Arg Tyr Ile Ala Val Lys Leu Phe Asn Tyr Asn Tyr Ser Leu Thr

370

375

380

Glu Tyr Gly Met His Trp Asn Phe Phe Leu Thr Leu Phe Phe Thr Leu

385

390

395

400

27 / 161

Leu Thr Cys Asn Ala Leu Leu Cys Leu Ile Arg Gly Val Lys Arg Thr

405

410

415

Phe His Leu Ser Cys Val Leu Ile Cys Leu Tyr Glu Ile Ile Ile Trp

420

425

430

Arg Leu Asp Ile Thr Ser Tyr Leu Val Val Asp Glu Ala Glu Arg Ser

435

440

445

Gly Phe Phe Ser Gln Asn Arg Glu Gly Leu Met Asn Val Ile Gly Ser

450

455

460

Val Asn Leu Tyr Leu Phe Ser Phe Ser Leu Trp Asn Gly Tyr Val Phe

465

470

475

480

Pro Asp Glu Gly Gln Gln Trp Glu Arg Gly Lys Ala Ala Arg Arg Pro

485

490

495

Asp Glu Ala Ala Arg Thr Pro Gly Glu Gly His Gly Gln Arg Ser Pro

500

505

510

Val Arg Leu Thr Leu Lys Leu Leu Ala Leu Ser Leu Leu Phe His Leu

515

520

525

Leu His Leu Leu Leu Asn Tyr Tyr Arg Asn Tyr Ser Val Arg Ile Leu

28 / 161

530

535

540

Cys Asn Ala Asn Tyr Ile Cys Val Val Ser Ser Val Ser Leu Phe Ala

545

550

555

560

Ala Ala Leu Ser Tyr Leu Val Glu Lys Val Leu Leu Arg Glu Lys Thr

565

570

575

Thr Thr Ile Pro Val Leu Gln Gln Met Asn Arg His Ser Leu Ala Val

580

585

590

Phe Leu Phe Cys Asn Val Thr Met Gly Thr Phe Asn Leu Leu Phe Gln

595

600

605

Ser Leu Leu Phe Pro Leu Phe Phe Ala Cys Leu Val Leu Ala Ala Tyr

610

615

620

Ser Tyr Gly Met Leu Arg Phe Ala Ser Leu Leu Pro Gly Pro Ala Gln

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630

635

640

Gly Glu Lys Gly Glu Lys Arg Glu Lys Gln Gln

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650

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<211> 2625

29 / 161

<212> DNA

<213> Artificial

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<223> an artificially synthesized sequence

<400> 5

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atctacggta aggagatccg taagaacgaa tccgcatact ctctacacta cgagaagtac	180
ctatacgaat tgtcacgaag atactacgag atcatcctga agtacaacaa ggagttggga	240
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aagcagaaga atagtacca ggtgaatac aataacgacg atgataacaa ttggaagttg	360
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aagaaggaca ttctgttgat ctacaagaat gagaagacga agtcctctat caagttcatt	480
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aagctggaca agaatgatta caacatcttg aagtctacca tcaacaattc caaggaaaac	600

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attatcaact ctgcatacat ttacatgtac attatcttct tcttcctgtg catatacgtc 660

gagaagaacc tgttcttgta cttcccaata ttgcttcaga agtatgagat tctcactacg 720

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atcaagctga tttgctcatg cttagtccta tacgtgactt tccagttgat ctactatacg 840

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tgcgatgaga aggaggaaat ctgtgatgaa aaggaagaga tttgcgatga gaaggaagag 960

atttgcatg agaaggaaga gatttgtgat gagaaggaag aaatctgcga tgagaaagaa 1020

gaaatccttg ataagaagaa gaagatccac gagaagaaga agaagatcca tgataagaaa 1080

gaggaaatcg atgagaagaa gaagaagatt catgacaaaa aggacgaaag tcatgataag 1140

aacgaggaca ttacgtatcc agtccagtac aatatcgaga atgacctatg gtattcatcc 1200

aagaacgtgg acatcaagat gtattcatcc agcaacaagg gtgaagaata cattatccag 1260

aacacgttga aacatttccg attgatgaac atgtgtatga cgtacatttg tatcttcgct 1320

gttgacttct acttcttccc taaccatttc tgcaagtcct actattacgg aaatacgttg 1380

31 / 161

atggacattg gaatcgggtgc atccatttct tccagtgcac actcgcagga gatcaagaag 1440

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ctgggaatta gcagatttat cggatatctac ctattcaact ataactacaa catttctgag 1560

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tgtttcatcc tcctcaagag gattcggtat atcttcctat tcagtattat ctgatcatc 1680

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acgtccaata tccctaagaa caagaaagat atgaataact caatgtattc gaagaatgga 1920

aaccatacta attcgaatat caacaaccgt aaccataaga tcgtcatccg taataaccac 1980

attaacaagt atgagcagga caacacgaac aagtacatca acaagcaaatt caataacaac 2040

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aagaacctca agaagaaaat caagtactat ctattgtacc ttcagtatat catcaacatc 2160

3 2 / 1 6 1

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cctaagaaga agacgcatca cgaagagaag cagaagaaag agtga 2625

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<211> 2010

<212> DNA

<213> Plasmodium falciparum

<400> 6

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atTTTtatatg atgtaaaaaa gtatgtgtat aatatggTTC atgatacacgt aaatacatta	180
gtcctagaag cTTTtagaag agaagacata ataaaaaaaa taaaagtaaa agaaaaacaa	240
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aaagaaaata aattaaaatt aaatgataag gatgaatata tttctttcaa ttttattgaa	660
gataaattaa ccgaatcatt tcatatgaat caaataattc atttaattaa taaaaaatgt	720
gtatttacca aatgcctaga aaattataaa aatagatatt ttgtactgaa aaaagaagag	780
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3 4 / 1 6 1

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3 5 / 1 6 1

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ggggaacatg aactgttaaa aaggtatccg actaacagct acttgttgct agaaaagatt 1860

cattttttat tttttgataa aataaaattg ttataaata ttttccttta ttttaaaaac 1920

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<212> PRT

<213> Plasmodium falciparum

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Met Cys Thr Thr Lys Asn Glu Asp Asn Asn Lys Val Asn Tyr Leu Tyr

1

5

10

15

Leu Ile Tyr Tyr Asn Asn Ile Pro Ile Phe Lys Lys Lys Ser Gly Asn

20

25

30

36 / 161

Glu Gln Asn Ile Ser Ala Leu Phe Ile Leu Tyr Asp Val Lys Lys Tyr

35

40

45

Val Tyr Asn Met Val His Asp His Val Asn Thr Leu Val Leu Glu Ala

50

55

60

Phe Arg Arg Glu Asp Ile Ile Lys Lys Ile Lys Val Lys Glu Lys Gln

65

70

75

80

Asn Asn Asn Asp Lys Asn Lys Glu Ser Asn Ile Glu Lys Asp Lys Asn

85

90

95

Glu Gln Thr Lys Phe Thr Asp Ile Tyr Asp Thr Asn Ser Lys Ser Asp

100

105

110

Lys Asp Ile Gln Lys Asn Asn Met Asn Asp Gly Asp Ser Asn Asn Lys

115

120

125

Asn Ser Ser Leu Phe Ile Asp Pro Phe Glu Ser Asp Ser Tyr Glu Lys

130

135

140

Asn Asn Phe Ser Asn Glu Lys Cys Ala Phe Gln Asn Val Asp Lys Ser

145

150

155

160

Lys Lys Asp Lys Glu His Ile Tyr Ser Glu Asn Ile Thr Pro Ser Ser

37 / 161

165

170

175

Ser Asn Asn Asn Asn Asp Asn Asn Lys Glu Asn Asp Cys Asp Lys Glu

180

185

190

Gln Leu Asp Lys Tyr Asn Lys Asp Lys Glu Asn Lys Leu Lys Leu Asn

195

200

205

Asp Lys Asp Glu Tyr Ile Ser Phe Asn Phe Ile Glu Asp Lys Leu Thr

210

215

220

Glu Ser Phe His Met Asn Gln Ile Ile His Leu Ile Asn Lys Lys Cys

225

230

235

240

Val Phe Thr Lys Cys Leu Glu Asn Tyr Lys Asn Arg Tyr Phe Val Leu

245

250

255

Lys Lys Glu Glu Ile Leu Lys Lys Lys Lys Lys Gln Lys Lys Met Ser

260

265

270

Ile Phe Ser Tyr Ile Val Ser Ile Ile Leu Phe Phe Thr Tyr Ile Ile

275

280

285

Ser Leu Ile Asn Ser Cys Leu Tyr Tyr Ile Ile Cys Thr Pro Lys Leu

290

295

300

38/161

Phe Ser Glu Tyr Ile Phe Ser Lys Lys Cys Asp Gly Tyr Leu Gln Asn
305 310 315 320

Ser Ala Tyr Pro Lys Phe Ile Phe Pro Ser Glu Trp His Asn Ile Phe
325 330 335

Arg Ser Phe Met Lys Asn Lys Gln Asn Pro Ser Glu Tyr Tyr Lys Tyr
340 345 350

Arg Glu Ile Leu Leu Ile Arg Ile Ile Asn Leu Ile Ile Asp Ile Phe
355 360 365

Leu Gly Phe Leu Ile Phe Leu Leu Leu Tyr Phe Asn Val Ile Asn Leu
370 375 380

His Tyr Ile Ser Glu Lys Ala Gln Ile Phe Tyr Gly Thr Ser Thr Leu
385 390 395 400

Thr Ser Ile Leu Gly Thr Leu Leu Gln Asn Pro Leu Gly Phe Lys Leu
405 410 415

Asn Asn Asn Phe Thr Ser Phe Ile Gly Ser Ile Leu Val Ser Ile Leu
420 425 430

Asp Lys Trp Asp Leu Phe Thr Asn Thr Ile Pro Val Asn Asn Ser Thr
435 440 445

39 / 161

Val Leu Asn Phe Val Gly Tyr Thr Ser Leu Leu Gly Phe Ser Phe Phe

450

455

460

Leu Ser Phe Val Ile Asp Tyr Leu Arg Phe Val Thr Ala His Val Thr

465

470

475

480

Ile Ile Tyr Leu Phe Leu Lys Lys Ile Cys Thr Leu Phe His Lys Asn

485

490

495

Met Tyr Ser Leu Tyr Leu Leu Phe Asn Gly Lys Lys Trp Asn Ile Leu

500

505

510

Lys Leu Arg Val Asp Thr Asn Tyr Tyr Ser Asn Glu Glu Val Leu Leu

515

520

525

Gly Thr Ile Leu Phe Thr Ile Leu Ile Phe Leu Tyr Pro Thr Ile Phe

530

535

540

Val Leu Val Leu Val Phe Gly Leu Ile Tyr Leu Ile Ile Asn Arg Ile

545

550

555

560

Ile Tyr Leu Leu Cys Val Met Glu Lys Ile Ile Leu Tyr Thr Pro Phe

565

570

575

Tyr Ile Phe Phe Ile Gln Pro Asn Cys Asn Lys Tyr Ile Ser Lys Gly

4 0 / 1 6 1

580

585

590

Phe Lys Phe Thr Lys Tyr Glu Val Gly Glu His Glu Leu Leu Lys Arg

595

600

605

Tyr Pro Thr Asn Ser Tyr Leu Leu Leu Glu Lys Ile His Phe Leu Phe

610

615

620

Phe Asp Lys Ile Lys Leu Phe Ile Asn Ile Phe Leu Tyr Phe Lys Asn

625

630

635

640

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Cys Leu Leu Lys Lys Lys Tyr Ile Tyr Ile Tyr Ile Ile

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665

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<211> 1482

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<213> Plasmodium falciparum

<400> 8

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41 / 161

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tatatgaata acaatgttat attattaagt acatcaagac attatittaa ttatagacat 240

accactaatt tattgattgc atataaatat cttaaattatt tcggtgatac tatggataag 300

aatattttat taatgattcc atttgatcaa gcttgtgatt gtaggaatat aagagaaggt 360

caaataattc gagaatatga attatttcct agtagtcata ataaagaaac aaaaatagaa 420

aatataaatt tatatgagaa tttaaattatt gattataaaa ataataatgt acgtgatgaa 480

caaattagaa gagtacttag acatagatat gatgctttta cacctaaaaa aaatagatta 540

tataataatg gaaataatga gaaaaattta tttctttata tgaccggaca tgggtggtgtg 600

aattttttta aaattcaaga atttaattatt attagttcct ctgaatttaa tatatatata 660

caagaattac ttatcaaaaa tttttataaa tatatatctg taattattga tacgtgtcaa 720

ggatatagtt tttatgatga tattctaaat ttgttatata aaaaaaaaaat taataatatc 780

ttctttttat catcttctaa aagaaatgaa aatagttata gtttattttc cagtagttat 840

4 2 / 1 6 1

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cataaaatat atgaaaaaga accatctaaa aatataaaag ccttttcatt atataacatt 960

ttaaattatt taaaaacaca acatattatg tcagaacctt ctacaaataa ttctaaattt 1020

aattcgtcca tttttttaca tgataaaaaat attcttttct tcaattctaa ttgttaatt 1080

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caaactctat atgaacaaac gttatattat aataataatc aacagaattt cttttctaatt 1260

catatgtcta attttacaga ttatTTTTTT acacatgata tatataatat atataatata 1320

tataatgtat ataatatata taatgtatat aatatatata atgtatatga tatatataat 1380

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<211> 493

<212> PRT

4 3 / 1 6 1

<213> Plasmodium falciparum

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1 5 10 15

Trp Val Cys Gly Ser Val Asn Phe Thr Gly Phe Asp Asn Lys Asn Met

20 25 30

Ile Gly Lys His Val Glu Leu Glu Gly Arg Tyr Lys Lys Glu Tyr Ile

35 40 45

Asp Arg Phe Phe Leu Glu Glu Leu Arg Lys His Asn Tyr Met Asn Asn

50 55 60

Asn Val Ile Leu Leu Ser Thr Ser Arg His Tyr Phe Asn Tyr Arg His

65 70 75 80

Thr Thr Asn Leu Leu Ile Ala Tyr Lys Tyr Leu Lys Tyr Phe Gly Asp

85 90 95

Thr Met Asp Lys Asn Ile Leu Leu Met Ile Pro Phe Asp Gln Ala Cys

100 105 110

Asp Cys Arg Asn Ile Arg Glu Gly Gln Ile Phe Arg Glu Tyr Glu Leu

115 120 125

4 4 / 1 6 1

Phe Pro Ser Ser His Asn Lys Glu Thr Lys Ile Glu Asn Ile Asn Leu

130

135

140

Tyr Glu Asn Leu Asn Ile Asp Tyr Lys Asn Asn Asn Val Arg Asp Glu

145

150

155

160

Gln Ile Arg Arg Val Leu Arg His Arg Tyr Asp Ala Phe Thr Pro Lys

165

170

175

Lys Asn Arg Leu Tyr Asn Asn Gly Asn Asn Glu Lys Asn Leu Phe Leu

180

185

190

Tyr Met Thr Gly His Gly Gly Val Asn Phe Leu Lys Ile Gln Glu Phe

195

200

205

Asn Ile Ile Ser Ser Ser Glu Phe Asn Ile Tyr Ile Gln Glu Leu Leu

210

215

220

Ile Lys Asn Phe Tyr Lys Tyr Ile Phe Val Ile Ile Asp Thr Cys Gln

225

230

235

240

Gly Tyr Ser Phe Tyr Asp Asp Ile Leu Asn Phe Val Tyr Lys Lys Lys

245

250

255

Ile Asn Asn Ile Phe Phe Leu Ser Ser Ser Lys Arg Asn Glu Asn Ser

4 5 / 1 6 1

260

265

270

Tyr Ser Leu Phe Ser Ser Ser Tyr Leu Ser Val Ser Thr Val Asp Arg

275

280

285

Phe Thr Tyr His Phe Phe Asn Tyr Leu Gln Gln Ile His Lys Ile Tyr

290

295

300

Glu Lys Glu Pro Ser Lys Asn Ile Lys Ala Phe Ser Leu Tyr Asn Ile

305

310

315

320

Leu Asn Tyr Leu Lys Thr Gln His Ile Met Ser Glu Pro Thr Thr Asn

325

330

335

Asn Ser Lys Phe Asn Ser Ser Ile Phe Leu His Asp Lys Asn Ile Leu

340

345

350

Phe Phe Asn Ser Asn Leu Leu Ile Ile His Lys Asp Asp Val Ser Ile

355

360

365

Ile Tyr Gln Asp Lys Gln Thr His Asn His Lys Tyr Ile Cys Leu Asp

370

375

380

Asn Leu Ser Lys Cys Gly His Ile Lys Asn Asn Val His Lys Lys Met

385

390

395

400

4 6 / 1 6 1

Gln Thr Leu Tyr Glu Gln Thr Leu Tyr Tyr Asn Asn Asn Gln Gln Asn

405

410

415

Phe Phe Ser Asn His Met Ser Asn Phe Thr Asp Tyr Phe Phe Thr His

420

425

430

Asp Ile Tyr Asn Ile Tyr Asn Ile Tyr Asn Val Tyr Asn Ile Tyr Asn

435

440

445

Val Tyr Asn Ile Tyr Asn Val Tyr Asp Ile Tyr Asn Val Tyr Ser Phe

450

455

460

Leu Ile Leu Leu Leu Ser Leu Phe Phe Ile Met Cys Ser Leu Leu Thr

465

470

475

480

Tyr Tyr Ile Val Phe Phe Thr Glu Lys Ala Lys Met Thr

485

490

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<211> 1494

<212> DNA

<213> Plasmodium falciparum

<400> 10

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47 / 161

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ggaggaatag aaactcacat ttttgaattg tctaagaatt tgataaaaaa gggtttcaag 180

gttatagttg taacaaattt taataataat aggcatggta taagatggat gggtaatggt 240

attaaggttt attatttgcc cttccagcct tttttagatg tagtgagttt tccaaatatt 300

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ggtcaccagg ctacgtcagc attagctcat caattcattc ttcatgcca aaccttagga 420

ataaaaacta tttatacaga tcactcatta tacagttttt cagacaaagg atgcatacat 480

gtaaacaaat tattgaaata ttgtataaac gatgttgatc attctatatg tgtttcccat 540

acgaatagag aaaatttagt ttgagaaca gaaagtaatc catataaaac gtcagttata 600

ggaaatgccc ttgatactac aaaatttggt ccttgtatta gtaaaagacc aaagtttcca 660

agaataaata ttattgttat aagtaggtta acatatagaa aaggatataga tttgatagtt 720

aaggtaatac cattagtatg tcaaaaatat ccattcataa aatttattat agggggagaa 780

ggtcctaaaa gattgttatt agaagaaatg agagaaaagt atcacttaca taattctgtt 840

48 / 161

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atttataatc taacccaaat gtactcttgg gaaaaagtag cggaaaagac ggtaaaatca 1260

cataatacat atattatgaa tattgaaaag gtgtatatga acgtattaaa ttatgcaaat 1320

ccaagcttgt ttaatagaat aagaaaaata tacgaaatta atacacctat tcatgtttcc 1380

tttttttttt ttttagacaa aatattgaag aggtcgtag ctttcctcat ttttatgatg 1440

acgaaaataa aaatgaaaaa taagagtttt aaaatgtcga tatataccac ataa 1494

<210> 11

<211> 497

49 / 161

<212> PRT

<213> Plasmodium falciparum

<400> 11

Met Glu Ser Ala Val Ser Glu Cys Asn Ile Tyr Lys Lys Glu Lys Asp

1 5 10 15

Lys Asn Ile Ile Tyr Lys Gln Glu Arg Lys Cys Cys Ile Cys Met Val

20 25 30

Ser Asp Phe Phe Tyr Pro Asn Leu Gly Gly Ile Glu Thr His Ile Phe

35 40 45

Glu Leu Ser Lys Asn Leu Ile Lys Lys Gly Phe Lys Val Ile Val Val

50 55 60

Thr Asn Phe Asn Asn Asn Arg His Gly Ile Arg Trp Met Gly Asn Gly

65 70 75 80

Ile Lys Val Tyr Tyr Leu Pro Phe Gln Pro Phe Leu Asp Val Val Ser

85 90 95

Phe Pro Asn Ile Ile Gly Thr Leu Pro Leu Cys Arg Asn Ile Leu Tyr

100 105 110

Arg Glu Lys Val Asp Ile Val His Gly His Gln Ala Thr Ser Ala Leu

50/161

115

120

125

Ala His Gln Phe Ile Leu His Ala Lys Thr Leu Gly Ile Lys Thr Ile

130

135

140

Tyr Thr Asp His Ser Leu Tyr Ser Phe Ser Asp Lys Gly Cys Ile His

145

150

155

160

Val Asn Lys Leu Leu Lys Tyr Cys Ile Asn Asp Val Asp His Ser Ile

165

170

175

Cys Val Ser His Thr Asn Arg Glu Asn Leu Val Leu Arg Thr Glu Ser

180

185

190

Asn Pro Tyr Lys Thr Ser Val Ile Gly Asn Ala Leu Asp Thr Thr Lys

195

200

205

Phe Val Pro Cys Ile Ser Lys Arg Pro Lys Phe Pro Arg Ile Asn Ile

210

215

220

Ile Val Ile Ser Arg Leu Thr Tyr Arg Lys Gly Ile Asp Leu Ile Val

225

230

235

240

Lys Val Ile Pro Leu Val Cys Gln Lys Tyr Pro Phe Ile Lys Phe Ile

245

250

255

51 / 161

Ile Gly Gly Glu Gly Pro Lys Arg Leu Leu Leu Glu Glu Met Arg Glu

260

265

270

Lys Tyr His Leu His Asn Ser Val Val Leu Leu Gly Lys Val Lys Gln

275

280

285

Glu Asn Val Lys Asn Ile Leu Gln Thr Gly His Ile Phe Leu Asn Thr

290

295

300

Ser Leu Thr Glu Ala Phe Cys Ile Ala Ile Ile Glu Ala Ala Ser Cys

305

310

315

320

Gly Leu Leu Val Ile Ser Thr Asp Val Gly Gly Ile Ser Glu Val Leu

325

330

335

Pro His Asp Met Met Ile Leu Ala Lys Pro Asn His Ile Glu Leu Cys

340

345

350

Lys Ala Val Asp Lys Ala Leu Lys Ile Val Gln Lys Val Asp Ser Asn

355

360

365

Leu Phe His Glu Arg Val Asn Met Ser Leu Leu Thr Tyr Val Asn Ile

370

375

380

Tyr Ile Tyr Ile Tyr Ile Tyr Ile Tyr Ile Ile Tyr Met Asn Asn Phe

385

390

395

400

5 2 / 1 6 1

Ile Tyr Asn Leu Thr Lys Met Tyr Ser Trp Glu Lys Val Ala Glu Lys

405

410

415

Thr Val Lys Ser His Asn Thr Tyr Ile Met Asn Ile Glu Lys Val Tyr

420

425

430

Met Asn Val Leu Asn Tyr Ala Asn Pro Ser Leu Phe Asn Arg Ile Arg

435

440

445

Lys Ile Tyr Glu Ile Asn Thr Pro Ile His Val Ser Phe Phe Phe Phe

450

455

460

Leu Asp Lys Ile Leu Lys Arg Ser Leu Ala Phe Leu Ile Phe Met Met

465

470

475

480

Thr Lys Ile Lys Met Lys Asn Lys Ser Phe Lys Met Ser Ile Tyr Thr

485

490

495

Thr

<210> 12

<211> 2361

<212> DNA

<213> Plasmodium falciparum

53/161

<400> 12

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54/161

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aaaataggta ttacgtacac ttttttttc ttaactatta ttattataat tgattcttat 1200

tattttgggc acattacatt tcccttttgg aatttttttg tttataattt ttttaagtga 1260

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5 5 / 1 6 1

gaacataaaa ttgttatagg atatataccg tttcttacaa tttttgttgg atatgcatta 1560

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5 6 / 1 6 1

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<211> 786

<212> PRT

<213> Plasmodium falciparum

<400> 13

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Leu Ile Asp Ser Lys Ile Phe Ile Trp Leu Leu Ile Phe Phe Arg Ile

20 25 30

Phe Asn Cys Leu Phe Val Val Thr Ser Phe Tyr Pro Asp Glu Tyr Phe

35 40 45

Gln Ser Val Glu Ile Ala His Phe Trp Ala Tyr Gly Tyr Gly His Met

50 55 60

Ser Trp Glu Trp Glu Pro Cys Val Ala Leu Arg Ser Val Ile Thr Pro

65 70 75 80

5 7 / 1 6 1

Phe Ile Tyr Tyr Val Leu Phe Leu Phe Leu Lys Leu Ile Asn Ile Asp

85

90

95

His Pro Val Cys Val Leu Tyr Ile Pro Lys Leu Cys His Gly Ile Cys

100

105

110

Ala Ala Leu Cys Asp Leu Gly Ile Tyr Lys Leu Leu Ile Tyr Trp Tyr

115

120

125

Val Glu Leu Tyr Asn Asp Ala Trp Ile Asn Glu Asp Asn Ile Lys Arg

130

135

140

Asn Glu Lys Asp Glu Asn Asn Gly Asn Asn Asn Asn Asn Asn Asn Asn

145

150

155

160

Asn Asn Asn Asn Asn Asn Asn Asn Asn Asn Tyr Tyr Tyr His Asn Asn

165

170

175

Ile Leu Tyr Asn Thr Asn Asp Ile Ile Ser Thr Ile Leu Cys Cys His

180

185

190

Phe Phe Cys Trp Phe Tyr Phe Tyr Ser Ile Cys Arg Thr Ser Ser His

195

200

205

Ser Phe Glu Cys Leu Phe Asn Ile Trp Gly Val Tyr Phe Leu Ser Gln

210

215

220

58/161

Asn Tyr Tyr Pro Leu Lys Asn Gln Ser Asn Lys Ile Glu Lys Ile Asp

225

230

235

240

Leu Leu Leu Gln Asn Asp Val Ile Ile Gln Lys Gly Lys Lys His Leu

245

250

255

Asn Glu Trp Thr Asn Leu Lys Glu Arg Arg Asn Asp His His Phe Asp

260

265

270

Thr Tyr Glu Asn Asn Phe Ile Tyr His Lys Gly Thr Gln Asn Cys Lys

275

280

285

Gln Tyr Asp Lys Asn Met Val Asp Gln Asn Val Cys Gly Gln Asn Met

290

295

300

Val Asp His Ile Ile Gln Asn Arg Asn Asn Leu Cys Arg Thr His Phe

305

310

315

320

Tyr Ser Ser Lys Phe Asn Lys Ile Gln Glu Ala Lys Asn Leu Leu Phe

325

330

335

Ser Leu Phe Phe Ser Ser Leu Ser Val Ile Phe Arg Pro Asn Ala Leu

340

345

350

Val Phe Trp Leu Ser Leu Tyr Ile Leu Tyr Ile Ile Lys Asn Ile Phe

59 / 16.1

355

360

365

Glu Lys Gln Asn Lys Ile Asn Tyr Lys Glu Ile Phe Lys Ile Gly Ile

370

375

380

Thr Tyr Thr Phe Phe Phe Leu Thr Ile Ile Ile Ile Ile Asp Ser Tyr

385

390

395

400

Tyr Phe Gly His Ile Thr Phe Pro Phe Trp Asn Phe Phe Val Tyr Asn

405

410

415

Phe Leu Ser Gly Asn Asn Lys Tyr Phe Gly Gly His Ser Phe Phe Phe

420

425

430

Tyr Phe Val Cys Val Ile Pro Ser Ile Tyr Leu Thr Leu Thr Pro Phe

435

440

445

Leu Phe Tyr Gly Tyr Tyr Ile Ile Tyr Asn Asn Ile Leu Asn Lys Val

450

455

460

Lys Tyr Lys Thr Ile Asn Ile Tyr Met Tyr Ile Leu Lys Arg Ile Asp

465

470

475

480

Trp Ile Val Tyr Leu Val Thr His Leu Glu Ile Leu Ser Leu Ser Phe

485

490

495

60/161

Ser Lys His Lys Glu His Lys Ile Val Ile Gly Tyr Ile Pro Phe Leu

500

505

510

Thr Ile Phe Val Gly Tyr Ala Leu Tyr Ile Ile Lys Leu His Tyr Lys

515

520

525

Lys Tyr Asn Gly Lys Asn Gly Lys Asn Ile Tyr Asn Asn Asn Lys Ile

530

535

540

Gln Tyr Gly Asn Ile Thr Ile Lys Gly Arg Asn Lys Tyr Ile Phe Leu

545

550

555

560

Ile Ser Ser Ser Leu Phe Thr Asn Ile Ser Phe Leu Leu Gln Phe Leu

565

570

575

Cys Ile Leu Phe Phe Cys Leu Ile His Asn Arg Ser Pro Glu His Val

580

585

590

Ala Ser Tyr Phe Arg Asn Leu Glu Thr Lys Asp Asp Gln Asn Ile Tyr

595

600

605

Ile Phe Ile Thr Asn Cys Tyr Asp Ile Pro Leu Tyr Ser His Ile His

610

615

620

Arg Lys Phe Asn Ile Gly Phe Leu Asp Cys Ser Pro Tyr Asp Thr Ser

625

630

635

640

61 / 161

Asn Asp Glu Ala Thr Lys Asn Trp Arg Lys Arg Ile Tyr Glu Asp Lys

645

650

655

Phe Lys Glu Gln Phe Phe Asn Ile Phe Gln Glu Lys Lys Asn Asn Asn

660

665

670

His His Ile Asn Ser Thr Tyr Gly Asp Thr Ile Thr Pro Tyr Ile Ile

675

680

685

Pro Asp Lys Ser Phe Tyr Trp Phe Gly His His His Phe Asn Lys Lys

690

695

700

Asn Asn Phe Gln Tyr Ile Tyr Gln Asn Ile Asn Leu Ser Cys Leu Asn

705

710

715

720

Tyr Arg Phe His Ile Pro Leu Gln Gly Gln Leu Pro Thr Tyr Ile Val

725

730

735

Thr Thr Thr Ile Glu Leu Thr His Leu Gln Leu Phe Leu Ser Thr Tyr

740

745

750

Asn Tyr Lys Leu Glu Thr Lys Pro Phe Phe Ser Tyr Phe Met Ile Ser

755

760

765

Glu Ala Lys Gly Ile Val Pro Val Tyr His Tyr Ile Phe Lys Arg Val

6 2 / 1 6 1

770

775

780

Pro Ser

785

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<213> Plasmodium falciparum

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tcatttatcg atattcttgt tactattcct ataaatcaaa ttataaaaat caaatatact 360

aattgtaaaa attatatatt ttatacttgt ttatggtttt taaatccatt agtcataatt 420

6 3 / 1 6 1

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1233

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<212> PRT

<213> Plasmodium falciparum

<400> 15

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1 5 10 15

Phe Phe Glu Trp Leu Ile Phe Phe Val Gly Ile Ile Ile Arg Ile Ile

20 25 30

Ile Tyr Tyr Tyr Gly Arg Trp Gln Asp Lys Asn Phe Asn Val Lys Phe

35 40 45

Thr Asp Val Asp Tyr Tyr Val Phe Ser Asp Ala Ala Lys Tyr Val Leu

50 55 60

Met Asn Lys Ser Pro Tyr Glu Arg Tyr Thr Tyr Arg Tyr Thr Pro Leu

65 70 75 80

Leu Ala Tyr Ile Met Ile Pro Asn Phe Phe Val His Phe Ser Phe Gly

85 90 95

65 / 161

Lys Ile Leu Phe Ser Phe Ile Asp Ile Leu Val Thr Ile Leu Ile Asn

100

105

110

Gln Ile Ile Lys Ile Lys Tyr Thr Asn Cys Lys Asn Tyr Ile Phe Tyr

115

120

125

Thr Cys Leu Trp Phe Leu Asn Pro Leu Val Ile Ile Ile Ser Leu Arg

130

135

140

Gly Asn Ala Asp Val Ile Pro Cys Phe Leu Ile Ile Val Thr Ile Phe

145

150

155

160

Cys Ile Tyr Lys Lys His Ile Phe Leu Ser Ser Ile Phe Tyr Gly Leu

165

170

175

Ala Val Asn Phe Lys Ile Tyr Thr Ile Ile Tyr Ala Leu Pro Phe Met

180

185

190

Leu Tyr Leu Asn Lys Asn Tyr Leu Leu Gly Glu Asn Ile Phe Gln Leu

195

200

205

Asn Glu Lys Lys Lys Lys Lys Lys Asn Asp Phe Leu Leu Asn Thr Phe

210

215

220

Phe Tyr Ile Phe Arg Ile Ile Ser Asn Phe Phe Val Glu Leu Phe Lys

6 6 / 1 6 1

225 230 235 240

Leu Asn Tyr Glu Gln Phe Leu Phe Ala Ile Cys Ser Ser Ser Val Phe

245 250 255

Leu Ile Leu Asn Cys Val Phe Tyr Ile Ile Tyr Gly Tyr Glu Phe Leu

260 265 270

Tyr Glu Ser Phe Ile Tyr His Ile Ile Arg Arg Asp His Arg His Asn

275 280 285

Phe Ser Leu Phe Phe Tyr Leu Met Tyr Leu Ser Ile Glu Lys Asn Ser

290 295 300

Lys Ile Ile Pro Leu Ile Thr Phe Val Pro Gln Ile Ile Leu Val Ala

305 310 315 320

Leu Phe Gly Phe Lys Tyr Ala Arg Thr Asn Leu Glu Leu Ser Met Phe

325 330 335

Leu Gln Thr Ile Ser Phe Ile Ala Leu Asn Lys Val Cys Thr Ser Gln

340 345 350

Tyr Phe Ile Trp Cys Ile Pro Phe Leu Pro Ile Ile Leu Cys Ala Ile

355 360 365

67/161

Thr Leu Ser Lys Arg Asn Met Phe Leu Ile Ile Ser Ser Ile Leu Phe

370

375

380

Phe Ile Val Ala Lys Val Gly Ser Lys Arg Phe Leu Leu Leu Tyr Lys

385

390

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Tyr Val Phe Ser Tyr Ile Ile Phe Phe Ser

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410

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<213> Plasmodium falciparum

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tcgattatta atgcaccata tgataaagtt gttatacttt taatagattc cttAagattt 300

68 / 161

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acgactagtg ttgaagataa ttttttgaa cagcttcatt taaataataa aaaagtaatt 660

gctatcgggtg ataataccat tactcatttg atgaaacatt tttctaaaga attagtttat 720

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6 9 / 1 6 1

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ttatggcaat tgaatagata cctgaatgaa tatgaaaaaa agtataatat aataaaaaat 1860

70 / 161

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tcatatatag aattcataaa tgaaatgaca actctaattg atatcacaca aaaatatattt 2040

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71 / 161

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gataaagaaa gatattatat tcattacatt ttaattgtat atgttatatt tgggatgctt 2760

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7 2 / 1 6 1

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<213> Plasmodium falciparum

<400> 17

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15

7 3 / 1 6 1

Phe Ile Ser Asn His Thr Leu Ile Phe Ser Ile Ile Leu Leu Ile Asn

20

25

30

Leu Leu Ile Phe Phe Ser Phe Ile Asn Gly Tyr Phe Tyr Ala Arg Gln

35

40

45

Lys Leu Glu Glu Lys Ser Glu Asn Leu Glu Leu Phe Ser Arg Lys Val

50

55

60

Phe Gly Asp Glu Tyr Val Glu Ser Leu Lys Lys Lys Lys Asn Thr Phe

65

70

75

80

Ser Ile Ile Asn Ala Pro Tyr Asp Lys Val Val Ile Leu Leu Ile Asp

85

90

95

Ser Leu Arg Phe Asp Phe Thr Leu Tyr Asp Thr Asn Tyr Glu Lys Glu

100

105

110

Phe Ile Gly Lys Glu Lys Asn Thr Asp Ile Tyr Asn Asn Ile Ser Ser

115

120

125

Glu Lys Lys Asn Ile Ser Asn Asp Gly Glu Lys Lys Asn Ser Leu Phe

130

135

140

Phe Leu Asn Asn Met Ile Asn Val His His Ile Leu Gln Asn Glu Lys

145

150

155

160

74/161

Asn Asn Thr Leu Leu Phe Arg Phe Asp Ala Asp Ala Pro Thr Ile Thr

165

170

175

Thr Ser Arg Ile Lys Ser Ile Phe Met Gly Thr Ile Pro Asn Tyr Met

180

185

190

Glu Val Asn Glu Asn Phe Ser Pro Thr Thr Ser Val Glu Asp Asn Phe

195

200

205

Phe Glu Gln Leu His Leu Asn Asn Lys Lys Val Ile Ala Ile Gly Asp

210

215

220

Asn Thr Ile Thr His Leu Met Lys His Phe Ser Lys Glu Leu Val Tyr

225

230

235

240

Glu Ser Phe Asn Val Phe Asp Phe Tyr Ser Leu Asp Ile Ala Ala Lys

245

250

255

Lys His Phe Tyr Glu Glu Tyr Glu Ser Asn Asp Trp Asp Ile Met Tyr

260

265

270

Ile His Met Leu Gly Val Asp His Ile Gly His Ile Lys Thr Pro Asn

275

280

285

Ser Lys Ile Met Gly Asp Ala Leu Lys Asp Phe Asp Thr Phe Ile Tyr

7 5 / 1 6 1

290	295	300	
Asp Ile Ile Asn Lys Ile Lys Leu Asp Asn Leu Lys Asn Ile Ser Thr			
305	310	315	320
Glu Glu Lys Glu Lys Met Lys Ile Ile Lys Lys Lys Ile Tyr Lys Thr			
	325	330	335
Tyr Arg Asn Gly His His Ile Gln Asn Glu Asn Asp His Ile Ile Asp			
	340	345	350
Asn Ile Gln Asn Glu Asn Asp His Ile Ile Asp Asn Ile Gln Asn Glu			
	355	360	365
Asn Asp His Thr Ile Asp Asn Ile Gln Ser Glu Asn Asp His Thr Ile			
	370	375	380
Asp Asn Ile Gln Asn Glu Asn Asp His Thr Ile Glu Asp Ile Gln Ile			
385	390	395	400
Asp Asn Asp His Thr Ile Glu Asp Ile Glu Asn Gln Ser Glu Gln Lys			
	405	410	415
Asn Asp Asp Lys Lys Thr Leu Phe Ile Phe Phe Gly Asp His Gly Gln			
	420	425	430

7 6 / 1 6 1

Leu Asp Thr Gly Asp His Gly Gly Tyr Ser Leu Asp Glu Thr His Ser

435

440

445

Ala Leu Phe Ala Tyr Ser Pro Leu Asn Phe Ile Ser Leu Asp Asn Asp

450

455

460

Ile Ile Gln Asn Asn Phe Val Leu Tyr Asp Lys Asp Lys Leu Lys Lys

465

470

475

480

Asn Val Asn Thr Leu Asn Glu Glu Asn Asn Asn Asn Glu Asn Ile Asp

485

490

495

Asn Tyr Lys Lys Tyr His Ser Tyr Leu Lys Asp Arg Asn Lys Lys Tyr

500

505

510

Ser Tyr His Tyr Asn Val Lys Tyr Thr Lys Gln Val Asn Leu Met Ser

515

520

525

Thr Leu Ser Leu Leu Ile Gly Ser Thr Leu Pro Tyr Gly Asn Ile Gly

530

535

540

Asn Ile Ile Met Asp Phe Ile Pro Asn Ala Tyr Ile Lys Asn Asn Asn

545

550

555

560

Lys Lys Lys Asn Asn Asn Asn Asn Asn Asn Asn Asn Ser Ser Leu

565

570

575

77/161

Pro Asn Glu Gln Thr Asn Leu Tyr Tyr Asp Leu Leu Asn Leu His Tyr

580

585

590

Ile Ala Glu Leu Asn Tyr Ala Asn Leu Trp Gln Leu Asn Arg Tyr Leu

595

600

605

Asn Glu Tyr Glu Lys Lys Tyr Asn Ile Ile Lys Asn Glu Asp Tyr His

610

615

620

Phe Ile Lys Ser Ser Trp His Ile Ile Gln Lys Asp Lys Lys Glu Leu

625

630

635

640

Phe Phe Gln Pro Asn Lys Lys Phe Ile Lys Asn Asp Ile Leu Leu Lys

645

650

655

Lys Glu Lys Glu Ser Tyr Ile Glu Phe Ile Asn Glu Met Thr Thr Leu

660

665

670

Met Asp Ile Thr Gln Lys Tyr Phe Tyr Tyr Ile Phe Asn Ile Lys Glu

675

680

685

Lys Tyr Phe Leu Ile Leu Ser Ile Val Leu Asn Ile Phe Leu Leu Leu

690

695

700

Phe Leu Lys His Phe Tyr Tyr Tyr Ser Lys Leu Asn Tyr Tyr His Lys

78/161

705 710 715 720

Leu Ile Lys Val Thr Phe Asn Asp Phe Asn Lys Asn Ile Tyr Leu Leu

725 730 735

Leu Cys Ile Cys Ala Leu Leu Leu Tyr Phe Phe Ile Phe Cys Leu Ser

740 745 750

Ile Lys Glu Tyr Lys Asp Ile Phe Arg Ile Phe Ser His Ala Lys Ile

755 760 765

Ile Phe Ile Ser Asn Asn Ile Asp Met Ile Ile Pro Ser Ile Lys Asn

770 775 780

Tyr His Met Ser Ala Lys Arg Asn Met Asn Ile Thr Asn Asn Asp Thr

785 790 795 800

Tyr His Thr Ser His Lys Asp Arg Lys Ser Phe Thr Asn Lys Glu Glu

805 810 815

Lys Gln Asn Asn Thr Leu Met Asn Ile Phe Tyr Asn Ile Ile Tyr Phe

820 825 830

Leu Arg Ile Ile Arg Lys Lys Ile Val Gln Tyr Ile Ile Phe Ile His

835 840 845

79 / 161

Leu Thr Ile Tyr Asn Leu Thr Ile Gly Asn Ile Ile Phe Ile Leu Phe

850

855

860

Lys Leu Phe Pro Lys Ile Ile Thr Asn Ser Phe Gln Ile Leu Arg Ser

865

870

875

880

Asn Tyr Phe Leu Leu Phe Val Ile Ile Trp Ser Cys Cys Glu Met Ser

885

890

895

Phe Asn Tyr Ile Asp Lys Glu Arg Tyr Tyr Ile His Tyr Ile Leu Ile

900

905

910

Val Tyr Val Ile Phe Gly Met Leu Lys Trp Lys Tyr His Arg Val Phe

915

920

925

Asn Ile Leu Lys Ala Phe Ile Leu Leu Val Leu Leu Ile Ile Asn Ala

930

935

940

Leu Tyr Ser His Thr Pro Glu Tyr Phe Asp His Gly Lys Glu Lys Ile

945

950

955

960

Tyr Leu Lys Glu Ser Val Leu Lys Ser Val Phe Pro Ile Ser Ser Tyr

965

970

975

Ile Leu Ser Leu Ile Leu Ile Asn Ser Gly Ile Asn Asn Leu Leu Lys

980

985

990

80 / 161

Lys Arg Ile Lys Ile Ile Ile Thr Gln Ile Trp Thr Leu Gln Tyr Ile

995

1000

1005

Leu Val Phe Leu Phe Leu Asn Asn Ile Tyr His Arg Tyr Ile Gln

1010

1015

1020

Phe Ile Thr Pro Pro Ser Ile Tyr Phe Leu Thr Ile Ser Thr Phe

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1035

Ile Phe Ile Phe Asn Thr Asn Leu Gly Val Leu Phe Leu Phe Tyr

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1045

1050

Met Thr Phe Leu Phe Phe Tyr Phe Ile Leu Ile Ser Ser Asn Cys

1055

1060

1065

Ser Glu Asn Met Ile Gln Met Asn Asp Ile Thr Ser Thr Trp Ile

1070

1075

1080

Asn Glu Asn Ile His Asn Arg Asn Asp Pro Ile Ile Thr Lys Gly

1085

1090

1095

Asn Leu Glu Asn Lys Glu Lys Cys Thr Ser Cys Asn Thr Ser Ile

1100

1105

1110

Lys Glu Lys Phe Tyr Tyr Lys Leu Met Ile Glu Lys Phe Lys Leu

81 / 161

1115

1120

1125

Leu Asn Thr Cys Ser Val Leu Asn Glu Glu Glu Ile Met Gly Ile

1130

1135

1140

Gln Ile Tyr Lys Leu Ile Arg Asp Ile Ser Tyr Phe Tyr Ile Asn

1145

1150

1155

Glu Thr Asp Phe Tyr Ile Leu Ser Cys Val Leu Leu Ile Tyr Ser

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1170

Phe Phe Ile Thr Gly His Lys Phe Ile Leu Asn Asn Leu Pro Leu

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Val Ser Gly Tyr Val Gly Leu Tyr Lys Tyr Val Trp Pro Ile Ser

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Gln Phe Tyr Ile Phe Asn His Ile Phe Phe Pro Phe Phe Phe Ser

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1230

Ile Asn Ser Phe Lys Gln Phe Asp Leu Tyr Tyr Phe Tyr Val Tyr

1235

1240

1245

8 2 / 1 6 1

Pro Leu Met Asn Phe Ser Phe Lys Ala Ser Phe Leu Phe Cys Cys

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Ile Met Val Lys Ile

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8 3 / 1 6 1

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84 / 161

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8 5 / 1 6 1

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<211> 810

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<213> Plasmodium falciparum

8 6 / 1 6 1

<400> 19

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Ile Lys Lys Ser Lys Val Ile Gly Val Phe Leu Ser Ile Ile Gly Val

20 25 30

Ile Phe Phe Leu Val Phe Asn Lys Phe Asn Lys Asn Ala Glu Leu Asp

35 40 45

Ala Arg Thr Phe Thr Gln Phe Val Gly Asn Ser Val Leu Asn Lys Lys

50 55 60

Asn Glu Lys Phe Tyr Asn Asp Thr Asn Ser Tyr Phe Met Asn Tyr Thr

65 70 75 80

Tyr Glu Gly Lys Glu Asp Ile Ile Lys Leu Ile Tyr Asp Tyr Ile Arg

85 90 95

Lys Asn Ile Leu Val Asn Val Glu Asn Glu Met Val Lys Ile Lys Leu

100 105 110

Thr Asp Arg Ile Glu Gln Asn Ile Leu Ile Ser Asn Val Gly Cys Lys

115 120 125

Tyr Cys Asn Asn Met Glu Ser Leu Val Val Val Ile Asn Phe Asp Phe

$$87 \div 161$$

130	135	140
Lys Glu Arg Lys Tyr Phe His Ser Val Ile Ile Gly Leu Thr Leu Met		
145	150	155
Glu His Phe Ser Lys Cys Asn Tyr Met Ser Lys Asp Val Thr Phe Leu		
	165	170
		175
Phe Thr Asn Lys Glu Leu Leu Tyr Ser Leu Gly Val Gln Glu Phe Ile		
	180	185
		190
Gln Lys Tyr Phe Tyr Asn Asn Thr Asn Arg Ile Gly Lys Lys Ile Ile		
	195	200
		205
Arg Ser Ser Thr Ile Ile Glu Phe Asp Ser Ile Tyr Pro Ser Tyr Ile		
	210	215
		220
Lys Ile Asn Tyr Glu Gly Leu Asn Gly Met Leu Pro Asn Gln Asp Leu		
225	230	235
		240
Ile Leu Leu Leu Thr Asn Glu Leu His Phe Tyr Ser Ile Pro Ile Lys		
	245	250
		255
Met Glu Leu Thr His Gly Ser Ile Phe Asp Met Ala Leu Glu Lys Asn		
	260	265
		270

88/161

Tyr Glu Asn Gly His Ile Tyr Phe Leu Arg Tyr Lys Lys Lys Tyr Glu

275

280

285

Tyr Ile Arg Asp Asp Asn Asp Glu Ile Lys Asn Ile Pro Ala Phe Thr

290

295

300

Ala Thr Gly Gly Ser Lys Val Pro Ile Arg Asn Lys Met Ile Asn Leu

305

310

315

320

Phe Asn Leu Thr Lys Ala Leu Gln Ser Tyr Leu Arg Ser Gln Ser Asn

325

330

335

Thr His Glu Gly Phe Cys His Ser Ser Asn Phe Tyr Phe Phe Asn Thr

340

345

350

Phe Arg Arg His Ile Pro Ile Ser Ile Tyr Cys Tyr Ser Val Tyr Leu

355

360

365

Ile Cys Ala Tyr Ser Ile Met Lys Leu Phe Lys Ser Thr Ile Phe Arg

370

375

380

Ser Tyr Ile Asn Phe Leu Thr Gly Phe Tyr Thr Tyr Leu Ile Thr Ile

385

390

395

400

Leu Ile Ile Ser Leu Pro Ile Tyr Leu Ile Ser Thr Asn Lys Lys Phe

405

410

415

89 / 161

Tyr Glu Leu Leu Asn Phe Glu Glu Asn Tyr Ile Pro Ser Cys Tyr Glu

420

425

430

Trp His Pro Asp Asn Phe Asp Lys Tyr Ile Lys Ile Ala Asn Ile Trp

435

440

445

Trp Asn Val Leu Phe Phe Ser Ile Phe Gly Ala Phe Phe Phe Asn Leu

450

455

460

Phe Ile Ser Phe Leu Val Asn Lys Lys Arg Lys Val Ile Pro Lys Lys

465

470

475

480

Asn Asp Gln Asn Glu Ser Phe Asp Gly Tyr Lys Lys Val Glu Lys Val

485

490

495

Glu Arg Ile Leu Ile Leu Glu Lys Ile Lys Glu Leu Gln Asn Glu Ile

500

505

510

Met Lys Arg Lys Gly Ile Thr Asn Asn His Asn Asn Ile Lys Asn Tyr

515

520

525

Asn Ile Tyr Thr Asn Glu Asn Ile Tyr Asn Asn Asn Ile Asn Asn Ile

530

535

540

Asn Asn Asn Asn Asn Ile Tyr Glu Asn Leu Tyr Asp Asn Gly Glu Val

90/161

545

550

555

560

Lys Lys Asn Ile Leu Val Lys Pro Lys Ile Ile Asn Ser Asp Asp Glu

565

570

575

Asp Phe Leu Leu Glu Lys Lys Asn Ser Glu Phe Ile Lys Lys Ile Glu

580

585

590

Lys Gln Ile Glu Ile Leu Glu Glu Lys Leu Glu Phe Leu Ser Asn Asp

595

600

605

Glu Asn Val Lys Tyr Ile Phe Tyr Asn Asn Ser Ile Ala Pro Phe Asn

610

615

620

Thr Met Met Ile Tyr Met Asn Ile Phe Tyr Phe Ile Leu Val Ala Leu

625

630

635

640

Leu Ser Ser Leu Tyr Asn Trp Ser Tyr Ser Val Leu Phe Ser Leu Leu

645

650

655

Phe Val Ile Pro Ile Ser Ile Leu His Asn Leu Lys Thr Lys Pro Val

660

665

670

Arg Ile Phe Lys Lys Ile Ile Leu Ser Leu Phe Ile Leu Cys Met Phe

675

680

685

91 / 161

Ile Tyr Met Tyr Pro Asn Asp Asn His Leu Trp Asn Ile Arg Gln Lys

690

695

700

Leu Thr Asn Leu Phe Arg Asn Asn Ile Ser Lys Cys Cys Lys Tyr Leu

705

710

715

720

Asp Lys His Lys Ile Leu Gln Ser Lys Tyr Phe Pro Glu Ser Leu Gln

725

730

735

Phe Ile Cys Ser Asn Arg Leu Phe Asp Ser Phe Tyr Ser Asn Lys Tyr

740

745

750

Phe Leu Asp Asn Leu Asn Ile Lys Phe Ser Tyr Val Leu Asp Ile Gln

755

760

765

Asn Gly Phe Leu Leu Thr Leu Tyr Asn Leu Ala Arg Asn His Phe Cys

770

775

780

Ile Gly Thr Ala Thr Tyr Pro Leu Ile Cys Phe Thr Leu Phe Pro Ile

785

790

795

800

Val Phe Tyr Ile Val Phe Leu Phe Phe Cys

805

810

9 2 / 1 6 1

<211> 780

<212> DNA

<213> Plasmodium falciparum

<400> 20

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tatcttattt atatgataat tgatgaatta aataaacatg aaattaaatt tgaaataatt 180

gtaatagatg ataatagtca agatgggtact gcagatgtgt acaaaaagtt acaaaacatt 240

tttaaggatg aagaattatt attaatacaa agaaaaggaa aattagggtt aggttctgca 300

tatatggaag gtttaaaaaa tgtaacagga gattttgtta taataatgga tgctgattta 360

tcacatcatc ctaaataatat ttataacttt attaaaaaac aaagagaaaa aaattgtgac 420

attgttacag gcacaagata taagaaccaa ggtggaatat caggatggtc atttaataga 480

attataataa gtagagtagc aaatttttta gctcaatttc tattattcat taatctatca 540

gatttaaccg ggtcttttag attatataaa actaatgtac tgaaggaaact tatgcaatct 600

attaataata caggttatgt ttttcaaatg gaagttcttg taagagcata taaatggga 660

9 3 / 1 6 1

aaatctatag aagaagttgg ttacgttttt gttgatagat tatttggaat atcaaaactg 720

gaaactacag atattttaca atacttatca ggtcttttca agttattctg gtcaatataa 780

<210> 21

<211> 259

<212> PRT

<213> Plasmodium falciparum

<400> 21

Met Val Ile Arg Phe Phe Leu Phe Val Ile Thr Leu Leu Gly Leu Cys

1

5

10

15

Ile Asn Met Val Cys Cys Asn Phe Lys Tyr Ser Ile Ile Leu Pro Thr

20

25

30

Tyr Asn Glu Lys Glu Asn Leu Pro Tyr Leu Ile Tyr Met Ile Ile Asp

35

40

45

Glu Leu Asn Lys His Glu Ile Lys Phe Glu Ile Ile Val Ile Asp Asp

50

55

60

Asn Ser Gln Asp Gly Thr Ala Asp Val Tyr Lys Lys Leu Gln Asn Ile

65

70

75

80

94/161

Phe Lys Asp Glu Glu Leu Leu Leu Ile Gln Arg Lys Gly Lys Leu Gly

85

90

95

Leu Gly Ser Ala Tyr Met Glu Gly Leu Lys Asn Val Thr Gly Asp Phe

100

105

110

Val Ile Ile Met Asp Ala Asp Leu Ser His His Pro Lys Tyr Ile Tyr

115

120

125

Asn Phe Ile Lys Lys Gln Arg Glu Lys Asn Cys Asp Ile Val Thr Gly

130

135

140

Thr Arg Tyr Lys Asn Gln Gly Gly Ile Ser Gly Trp Ser Phe Asn Arg

145

150

155

160

Ile Ile Ile Ser Arg Val Ala Asn Phe Leu Ala Gln Phe Leu Leu Phe

165

170

175

Ile Asn Leu Ser Asp Leu Thr Gly Ser Phe Arg Leu Tyr Lys Thr Asn

180

185

190

Val Leu Lys Glu Leu Met Gln Ser Ile Asn Asn Thr Gly Tyr Val Phe

195

200

205

Gln Met Glu Val Leu Val Arg Ala Tyr Lys Met Gly Lys Ser Ile Glu

9 5 / 1 6 1

210

215

220

Glu Val Gly Tyr Val Phe Val Asp Arg Leu Phe Gly Lys Ser Lys Leu

225

230

235

240

Glu Thr Thr Asp Ile Leu Gln Tyr Leu Ser Gly Leu Phe Lys Leu Phe

245

250

255

Trp Ser Ile

<210> 22

<211> 843

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 22

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acagatccaa gttttattga gttgagagca agacaaaagg ctgagagtaa ccagaagtct 120

gatagaaaat tatcagaagc tgctcgcgct caaattaggt tggattttat aagtttctac 180

caaaccatat tgaacacttc tttcatttac atcactttta catatattta ctattatggc 240

9 6 / 1 6 1

ttcgatccta ttccgccaac tattttcctt tcatttataa cattgattat atcaaggacg 300

aaagtcgacc ctctattgtc ctcatcctatg gacgtaaagt ctctcgtgat tatcacattt 360

gcaatgttga ctctctctcc agtcctcaaa tctctttcta aaacaactgc atctgattcc 420

atatggacat tgtctttttg gctgacccta tgggtacattt tcgttatttc gtcaacaaag 480

tccaaagata aaccctctaa cctttccacc aatatacttg tcgcccttgt tgctgtccta 540

tcctcgaggc ttctgaccac aatcgacgta ttctgttttc ttttaatttg tattcagttg 600

aatatcattc taccactta tttatcgggtg acgaataagg tagtaccaat aatttcaaat 660

attattgtat actcattttt gaatgttgct ctaggttgga tttatatgct gttgattttc 720

tttgcttcag tattttatat tactgtttta cctaagtggg tcactctactg gaaaatcaat 780

tatcataaac gggataacga tctactaagt acatgggatg caagaacacc aatattggat 840

tag 843

<210> 23

<211> 280

<212> PRT

97/161

<213> *Saccharomyces cerevisiae*

<400> 23

Met Thr Arg Ser Pro Trp Lys Arg Leu Leu Trp Leu Lys Gln Glu Tyr

1 5 10 15

Pro Asp Asn Tyr Thr Asp Pro Ser Phe Ile Glu Leu Arg Ala Arg Gln

20 25 30

Lys Ala Glu Ser Asn Gln Lys Ser Asp Arg Lys Leu Ser Glu Ala Ala

35 40 45

Arg Ala Gln Ile Arg Leu Asp Phe Ile Ser Phe Tyr Gln Thr Ile Leu

50 55 60

Asn Thr Ser Phe Ile Tyr Ile Thr Phe Thr Tyr Ile Tyr Tyr Tyr Gly

65 70 75 80

Phe Asp Pro Ile Pro Pro Thr Ile Phe Leu Ser Phe Ile Thr Leu Ile

85 90 95

Ile Ser Arg Thr Lys Val Asp Pro Leu Leu Ser Ser Phe Met Asp Val

100 105 110

Lys Ser Ser Leu Ile Ile Thr Phe Ala Met Leu Thr Leu Ser Pro Val

115 120 125

98/161

Leu Lys Ser Leu Ser Lys Thr Thr Ala Ser Asp Ser Ile Trp Thr Leu

130

135

140

Ser Phe Trp Leu Thr Leu Trp Tyr Ile Phe Val Ile Ser Ser Thr Lys

145

150

155

160

Ser Lys Asp Lys Pro Ser Asn Leu Ser Thr Asn Ile Leu Val Ala Leu

165

170

175

Val Ala Val Leu Ser Ser Arg Leu Ser Thr Thr Ile Asp Val Phe Cys

180

185

190

Phe Leu Leu Ile Cys Ile Gln Leu Asn Ile Ile Leu Pro Thr Tyr Leu

195

200

205

Ser Val Thr Asn Lys Val Val Pro Ile Ile Ser Asn Ile Ile Val Tyr

210

215

220

Ser Phe Leu Asn Val Ala Leu Gly Trp Ile Tyr Met Leu Leu Ile Phe

225

230

235

240

Phe Ala Ser Val Phe Tyr Ile Thr Val Leu Pro Lys Trp Phe Ile Tyr

245

250

255

Trp Lys Ile Asn Tyr His Lys Arg Asp Asn Asp Leu Leu Ser Thr Trp

99/161

260

265

270

Asp Ala Arg Thr Pro Ile Leu Asp

275

280

<210> 24

<211> 764

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 24

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aaccgaaagt tcaaaaaagt caagcaaat gggagggtag agattaacat gggcatacaa 180

taccacaaa ttgtacttat ttactactg aatattttgt tctatgtaat ttgcctaaga 240

tcaagatttc tcgaacatat taatagaact ttigaagtga caatcgcgcg aagtttccag 300

atcttaatta taatgggatt gtttgcctta ggtacaatta tacttgtgag gggacctagt 360

gtggaaactg taacaatttt caagaaagt ggactacagc tgtccagagt gaagggtatg 420

100/161

gttatatttc ctcaacaatg gaatcggaag ttctttgaac aagtagagtt tatatccaat 480

gaaagaatta ttgatgtagt gatcaatgaa ggattctgtc ggggatttcg agtgatattc 540

tatcttgcag caattgtacg taaatcgtct acgettaagc tattatttcc agtatgtatt 600

caagcgaatt taagattggt tcttacttca acgatctgat tagaaatact aacacaacaa 660

tgcagtcaaa ttgcccagt atcgatgacc aacgtctaata atacaacata tctagaaaat 720

atctcagtaa gcaagaaaaa cccctgagca gaccaaaga ttga 764

<210> 25

<211> 229

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 25

Met Ile Ser Lys Glu Tyr Glu Phe Gly Lys Thr Ser Ile Leu Asn Arg

1 5 10 15

Lys Lys Tyr Thr Leu Val Ile Asp Glu Asp Lys Asn Gly Asn Phe Ile

20 25 30

Arg Phe Thr Val Leu Pro Val Ser Asn Arg Lys Phe Lys Lys Val Lys

1 0 1 / 1 6 1

35

40

45

Gln Asn Gly Arg Val Glu Ile Asn Met Gly Ile Gln Tyr His Gln Ile

50

55

60

Val Leu Ile Leu Leu Leu Asn Ile Leu Phe Tyr Val Ile Cys Leu Arg

65

70

75

80

Ser Arg Phe Leu Glu His Ile Asn Arg Thr Phe Glu Val Thr Ile Ala

85

90

95

Arg Ser Phe Gln Ile Leu Ile Ile Met Gly Leu Phe Ala Leu Gly Thr

100

105

110

Ile Ile Leu Val Arg Gly Pro Ser Val Glu Thr Val Thr Ile Phe Lys

115

120

125

Glu Ser Gly Leu Gln Leu Ser Arg Val Lys Gly Met Val Ile Phe Pro

130

135

140

Gln Gln Trp Asn Arg Lys Phe Phe Glu Gln Val Glu Phe Ile Ser Asn

145

150

155

160

Glu Arg Ile Ile Asp Val Val Ile Asn Glu Gly Phe Cys Arg Gly Phe

165

170

175

1 0 2 / 1 6 1

Arg Val Ile Phe Tyr Leu Ala Ala Ile Val Arg Lys Ser Ser Thr Leu

180

185

190

Lys Leu Leu Phe Pro Ser Asn Leu Pro Ser Ile Asp Asp Gln Arg Leu

195

200

205

Ile Tyr Asn Ile Ser Arg Lys Tyr Leu Ser Lys Gln Glu Lys Pro Leu

210

215

220

Ser Arg Pro Lys Asp

225

<210> 26

<211> 423

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 26

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atcaacacgc tattggacat cttcccacaa agaagatgga ttattacctt ggagagcata 180

atgctgatgg gcatgctatg cacatacatc ggccttctga tgtacaatga agatacatta 240

1 0 3 / 1 6 1

acaccgccgc tagattctct atctacagta acggatgccg gtggtcaact tgtaatagag 300

gacgaccggc acgtattcgt taagaaatgg gcctttaaag aaacaagtgg tatttacgat 360

ctgtctctga tggatgcctg ccaacttctc tacctatatg ataacgacca taccagcaca 420

tag 423

<210> 27

<211> 140

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 27

Met Tyr Thr Lys Glu Tyr Tyr Trp Phe Ser Gln Tyr Met Ile Ile Thr

1 5 10 15

Ser Thr Leu Val Leu Thr Ile Ile Trp Ser Ile Leu Pro Ser Ser Leu

20 25 30

Gly Glu Ala Ala Pro Lys Gln Phe Ile Asn Thr Leu Leu Asp Ile Phe

35 40 45

Pro Gln Arg Arg Trp Ile Ile Thr Leu Glu Ser Ile Met Leu Met Gly

1 0 4 / 1 6 1

50

55

60

Met Leu Cys Thr Tyr Ile Gly Leu Leu Met Tyr Asn Glu Asp Thr Leu

65

70

75

80

Thr Pro Pro Leu Asp Ser Leu Ser Thr Val Thr Asp Ala Gly Gly Gln

85

90

95

Leu Val Ile Glu Asp Asp Pro Asp Val Phe Val Lys Lys Trp Ala Phe

100

105

110

Lys Glu Thr Ser Gly Ile Tyr Asp Leu Ser Leu Met Asp Ala Cys Gln

115

120

125

Leu Leu Tyr Leu Tyr Asp Asn Asp His Thr Ser Thr

130

135

140

<210> 28

<211> 915

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 28

atgaagatgt tgaggcgtac aaaggtaaatt ttttcaaaat tgctttacaa gattactaaa

60

105/161

ctagcaatag tactgacgat cctatatatt tattttacgc ccaaaatcgt ctcccgaac 120

aatgcatcat tgcagcatat ttttcctcat aaatatggcg attatgaaat caatttggtc 180

atagcgcacc ctgacgacga agttatgttt ttttccccca taattttctca actgaattcg 240

tactttccga gaaccgtccc atttaacata atctgcttat caaagggcaa cgccgaaggt 300

cttggcgaaa ccagggtaaag agaattaaat gactcgcccg ctttattgct acacaatgaa 360

agagcagtct ccgtacaggt gatggatttc caggatggta tggacgaaat atgggatatt 420

gattctataa cttcttctct ttcacaaaag atagatataa agaatcataa ctggaaccag 480

attatcgta cctttgattc atatggtgta tcaaatcata tcaaccacaa aagctgttat 540

gctgccgtta aaaagttggt ggatgattat gctcaacctt agaccaaaag aaatgaacaa 600

ccacctcatg tcaactgctt ttatttgaga agctacaaga acaacatcgt tttaaagtac 660

aactccttta tttgggaaat cctaaaaata ctttacgacc tgattttctcc attccgtaga 720

ataattcagg cgcttccgcc taacacagcc gccgaaaaag acaagctttc acttatgaat 780

acacatgcac aatacgtact agcgtttgcc actatgctaa atgctcacga atcccaagtt 840

1 0 6 / 1 6 1

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gtttatacat attag 915

<210> 29

<211> 304

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 29

Met Lys Met Leu Arg Arg Thr Lys Val Asn Phe Ser Lys Leu Leu Tyr

1 5 10 15

Lys Ile Thr Lys Leu Ala Ile Val Leu Thr Ile Leu Tyr Ile Tyr Phe

20 25 30

Thr Pro Lys Ile Val Ser Arg Asn Asn Ala Ser Leu Gln His Ile Phe

35 40 45

Pro His Lys Tyr Gly Asp Tyr Glu Ile Asn Leu Val Ile Ala His Pro

50 55 60

Asp Asp Glu Val Met Phe Phe Ser Pro Ile Ile Ser Gln Leu Asn Ser

65 70 75 80

1 0 7 / 1 6 1

Tyr Phe Pro Arg Thr Val Pro Phe Asn Ile Ile Cys Leu Ser Lys Gly

85

90

95

Asn Ala Glu Gly Leu Gly Glu Thr Arg Val Arg Glu Leu Asn Glu Ser

100

105

110

Ala Ala Leu Leu Leu His Asn Glu Arg Ala Val Ser Val Gln Val Met

115

120

125

Asp Phe Gln Asp Gly Met Asp Glu Ile Trp Asp Ile Asp Ser Ile Thr

130

135

140

Ser Ser Leu Ser Gln Lys Ile Asp Ile Lys Asn His Asn Leu Asn Gln

145

150

155

160

Ile Ile Val Thr Phe Asp Ser Tyr Gly Val Ser Asn His Ile Asn His

165

170

175

Lys Ser Cys Tyr Ala Ala Val Lys Lys Leu Val Asp Asp Tyr Ala Gln

180

185

190

Pro Lys Thr Lys Arg Asn Glu Gln Pro Pro His Val Thr Ala Leu Tyr

195

200

205

Leu Arg Ser Tyr Lys Asn Asn Ile Val Leu Lys Tyr Asn Ser Phe Ile

210

215

220

108/161

Trp Glu Ile Leu Lys Ile Leu Tyr Asp Leu Ile Ser Pro Phe Arg Arg

225

230

235

240

Ile Ile Gln Ala Leu Pro Pro Asn Thr Ala Ala Glu Lys Asp Lys Leu

245

250

255

Ser Leu Met Asn Thr His Ala Gln Tyr Val Leu Ala Phe Ala Thr Met

260

265

270

Leu Asn Ala His Glu Ser Gln Val Val Trp Phe Arg Tyr Gly Trp Trp

275

280

285

Ile Phe Ser Arg Phe Val Phe Val Asn Glu Phe Asp Val Tyr Thr Tyr

290

295

300

<210> 30

<211> 2760

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 30

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1 0 9 / 1 6 1

caaagtactc caacccctcc tgcaaagaga ttgtttttga ttgtcgggtga tggttttacgt 180

gcagatacca cttttgataa agtcactcat ccagtatccg gaaaaacaga atttctggca 240

ccttttatta gatctttgggt aatgaataat gccacctacg gtatatcaca taccagaatg 300

ccaactgaat ccggtcctgg tcatgttgct atgattgctg ggttttacga agatgttagt 360

gccgtcacia aaggttggaa gtcaaaccct gtcaatttcg atagtttttt caaccaatct 420

actcatactt attcattcgg ttcacctgac attttaccta tgttcaaaga tggegtttct 480

gacccaaata aagttgacac ttggatgtat gatcatactt tcgaggattt tacgcaatct 540

tccatcgagc tggatgcttt tgtctttaga cacttggatc aattattcca caattccaca 600

ctgaactcaa cattggatta tgaaattagg caagacggta atgtattctt tctacatcta 660

ctaggttgcg atactgccgg acattcttat agaccatatt ctgccgagta ttatgacaat 720

gtcaaatata ttgatgatca aatccctatc cttatagaca aagtcaacia gttttttgcg 780

gacgacaaaa ccgcatttat ttttacagca gatcatggta tgagtgcatt tggatcacat 840

ggtgacggtc atcctaacia cacaaggacc cctcttggtg cttgggggtgc gggtttgaat 900

110/161

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ggtgtgaact atcctaaaaa ttcagttggt gagttaccaa tagcatatat cgatggaaaa 1080

gaaagtgaca agcttgccgc attgtacaac aacgcaagaa gcattttaga gcagtactta 1140

gtcaagcaag atgaggtaat agactctcaa tttttttata aggaatactt caagtttggt 1200

gaaaagtctc attcacatta cttagaagag atagaaacct taattcagcg tatatctgaa 1260

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gagaatgtaa ttgatgacca aaaagcgtca ccattaagcc atgcagtatt tggttccata 1500

ggaattttac taaattggat tttgttctac caacattctc cgttcaattt ttacatgtac 1560

cttcttttcc cattatactt ttggagctat atttttacaa atagatccgt actacgttca 1620

ggtatcaagg aattcttcaa aggtacctct ccttgaaaa gagttttaat aacaatctct 1680

111/161

attatatcag tttatgaggg aattgtatat ggatttttcc atagatggac gtttacgcta 1740

attacaaata tattggcggt ttaccggttt atttgtgggg tgagagagct atccgtgaat 1800

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1 1 2 / 1 6 1

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accatttcca actattgttt ggcgatccta tcatctttgt tcatgcttat tttggaagta 2700

ctcggtcatg tgttgctaaa aaatgtcatc atacaggata aaaccaaaaa aacacaatag 2760

<210> 31

<211> 919

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 31

Met Trp Asn Lys Thr Arg Thr Thr Leu Leu Ala Val Gly Val Leu Phe

1 5 10 15

His Leu Phe Tyr Leu Trp Ser Ile Phe Asp Ile Tyr Phe Ile Ser Pro

20 25 30

Leu Val His Gly Met Ser Pro Tyr Gln Ser Thr Pro Thr Pro Pro Ala

35 40 45

1 1 3 / 1 6 1

Lys Arg Leu Phe Leu Ile Val Gly Asp Gly Leu Arg Ala Asp Thr Thr

50

55

60

Phe Asp Lys Val Thr His Pro Val Ser Gly Lys Thr Glu Phe Leu Ala

65

70

75

80

Pro Phe Ile Arg Ser Leu Val Met Asn Asn Ala Thr Tyr Gly Ile Ser

85

90

95

His Thr Arg Met Pro Thr Glu Ser Arg Pro Gly His Val Ala Met Ile

100

105

110

Ala Gly Phe Tyr Glu Asp Val Ser Ala Val Thr Lys Gly Trp Lys Ser

115

120

125

Asn Pro Val Asn Phe Asp Ser Phe Phe Asn Gln Ser Thr His Thr Tyr

130

135

140

Ser Phe Gly Ser Pro Asp Ile Leu Pro Met Phe Lys Asp Gly Ala Ser

145

150

155

160

Asp Pro Asn Lys Val Asp Thr Trp Met Tyr Asp His Thr Phe Glu Asp

165

170

175

Phe Thr Gln Ser Ser Ile Glu Leu Asp Ala Phe Val Phe Arg His Leu

1 1 4 / 1 6 1

180

185

190

Asp Gln Leu Phe His Asn Ser Thr Leu Asn Ser Thr Leu Asp Tyr Glu

195

200

205

Ile Arg Gln Asp Gly Asn Val Phe Phe Leu His Leu Leu Gly Cys Asp

210

215

220

Thr Ala Gly His Ser Tyr Arg Pro Tyr Ser Ala Glu Tyr Tyr Asp Asn

225

230

235

240

Val Lys Tyr Ile Asp Asp Gln Ile Pro Ile Leu Ile Asp Lys Val Asn

245

250

255

Lys Phe Phe Ala Asp Asp Lys Thr Ala Phe Ile Phe Thr Ala Asp His

260

265

270

Gly Met Ser Ala Phe Gly Ser His Gly Asp Gly His Pro Asn Asn Thr

275

280

285

Arg Thr Pro Leu Val Ala Trp Gly Ala Gly Leu Asn Lys Pro Val His

290

295

300

Asn Pro Phe Pro Val Ser Asp Asn Tyr Thr Glu Asn Trp Glu Leu Ser

305

310

315

320

1 1 5 / 1 6 1

Ser Ile Lys Arg Asn Asp Val Lys Gln Ala Asp Ile Ala Ser Leu Met

325

330

335

Ser Tyr Leu Ile Gly Val Asn Tyr Pro Lys Asn Ser Val Gly Glu Leu

340

345

350

Pro Ile Ala Tyr Ile Asp Gly Lys Glu Ser Asp Lys Leu Ala Ala Leu

355

360

365

Tyr Asn Asn Ala Arg Ser Ile Leu Glu Gln Tyr Leu Val Lys Gln Asp

370

375

380

Glu Val Ile Asp Ser Gln Phe Phe Tyr Lys Glu Tyr Phe Lys Phe Val

385

390

395

400

Glu Lys Ser His Ser His Tyr Leu Glu Glu Ile Glu Thr Leu Ile Gln

405

410

415

Arg Ile Ser Glu Gly Glu Asn Tyr Leu Glu Gln Glu Ala Ile Thr Leu

420

425

430

Thr Glu Glu Leu Met Gln Ile Thr Leu Glu Gly Leu His Tyr Leu Thr

435

440

445

Thr Tyr Asn Trp Arg Phe Ile Arg Thr Ile Val Thr Phe Gly Phe Val

450

455

460

1 1 6 / 1 6 1

Gly Trp Ile Phe Phe Ser Phe Ile Ile Phe Leu Lys Ser Phe Ile Leu

465

470

475

480

Glu Asn Val Ile Asp Asp Gln Lys Ala Ser Pro Leu Ser His Ala Val

485

490

495

Phe Gly Ser Ile Gly Ile Leu Leu Asn Trp Ile Leu Phe Tyr Gln His

500

505

510

Ser Pro Phe Asn Phe Tyr Met Tyr Leu Leu Phe Pro Leu Tyr Phe Trp

515

520

525

Ser Tyr Ile Phe Thr Asn Arg Ser Val Leu Arg Ser Gly Ile Lys Glu

530

535

540

Phe Phe Lys Gly Thr Ser Pro Trp Lys Arg Val Leu Ile Thr Ile Ser

545

550

555

560

Ile Ile Ser Val Tyr Glu Gly Ile Val Tyr Gly Phe Phe His Arg Trp

565

570

575

Thr Phe Thr Leu Ile Thr Asn Ile Leu Ala Phe Tyr Pro Phe Ile Cys

580

585

590

Gly Val Arg Glu Leu Ser Val Asn Ile Leu Trp Ile Ile Thr Ser Val

1 1 7 / 1 6 1

595

600

605

Leu Leu Ser Thr Phe Thr Leu Phe Asp Ala Val Lys Ile Glu Asp Leu

610

615

620

Asn Gln Ile His Leu Ala Gly Leu Leu Ile Ile Leu Ser Ala Phe Tyr

625

630

635

640

Ala Leu Tyr Lys Ile His Ser Arg Ile Asn Ser Tyr Thr Arg Ala Ile

645

650

655

Phe Ala Ile Gln Ile Ser Leu Val Ala Ala Met Leu Ala Val Thr His

660

665

670

Arg Ser Val Ile Ser Leu Gln Leu Arg Gln Gly Leu Pro Arg Glu Ser

675

680

685

Gln Val Ala Gly Trp Ile Ile Phe Phe Val Ser Leu Phe Val Met Pro

690

695

700

Ile Leu His Tyr Arg Lys Pro Asn Asn Asp Tyr Lys Val Arg Leu Leu

705

710

715

720

Ile Ile Tyr Leu Thr Phe Ala Pro Ser Phe Ile Ile Leu Thr Ile Ser

725

730

735

1 1 8 / 1 6 1

Phe Glu Ser Leu Phe Tyr Phe Leu Phe Thr Ser Tyr Met Val Gln Trp

740

745

750

Ile Glu Ile Glu Asn Lys Ile Lys Glu Met Lys Thr Gln Lys Asp Glu

755

760

765

Asn Trp Leu Gln Val Leu Arg Val Ser Val Ile Gly Phe Phe Leu Leu

770

775

780

Gln Val Ala Phe Phe Gly Thr Gly Asn Val Ala Ser Ile Ser Ser Phe

785

790

795

800

Ser Leu Glu Ser Val Cys Arg Leu Leu Pro Ile Phe Asp Pro Phe Leu

805

810

815

Met Gly Ala Leu Leu Met Leu Lys Leu Ile Ile Pro Tyr Gly Leu Leu

820

825

830

Ser Thr Cys Leu Gly Ile Leu Asn Leu Lys Leu Asn Phe Lys Asp Tyr

835

840

845

Thr Ile Ser Ser Leu Ile Ile Ser Met Ser Asp Ile Leu Ser Leu Asn

850

855

860

Phe Phe Tyr Leu Leu Arg Thr Glu Gly Ser Trp Leu Asp Ile Gly Ile

865

870

875

880

1 1 9 / 1 6 1

Thr Ile Ser Asn Tyr Cys Leu Ala Ile Leu Ser Ser Leu Phe Met Leu

885

890

895

Ile Leu Glu Val Leu Gly His Val Leu Leu Lys Asn Val Ile Ile Gln

900

905

910

Asp Lys Thr Lys Lys Thr Gln

915

<210> 32

<211> 660

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 32

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gtgtatgtga ggaaaacccc tctgatgaca tttccatacc atttagtagc actactttat 180

tactacgttt ttgtatcttc aaatttcaat acggtgaagt tgctaagttt tttgattcct 240

acacaagttg cttatttagt tttacaattc aataaatgca cagtttacgg taacaaaatc 300

1 2 0 / 1 6 1

attaagatca attactcatt gaccattatt tgtctagggtg ttacattttt gttgagcttt 360
 ccacaaatgt tattaactat attatttgggt gcgccattaa tggacttatt gtgggaaacc 420
 tggctgttgt cactgcattt tgcattttta gcataccctg cagtttattc tgtattttaat 480
 tgtgatttca aagtgggatt atggaagaag tattttatct ttatcgttgt aggggggttg 540
 attagttgtg ttgtcattcc tttggattgg gatagagatt ggcagaattg gccaatcct 600
 attgttggtg gaggttattt gggcgctttg gtgggctata ctatcggtgc ctatatataa 660

<210> 33

<211> 219

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 33

Met Pro Ala Lys Lys Arg Thr Arg Lys Thr Val Lys Lys Thr Val Ser

1 5 10 15

Phe Ser Asp Asp Thr Thr Leu Thr Thr His Gln Asn Arg Glu Lys Lys

20 25 30

1 2 1 / 1 6 1

Asn Val Asp His Asp Arg Pro Pro Val Tyr Val Arg Lys Thr Pro Leu

35

40

45

Met Thr Phe Pro Tyr His Leu Val Ala Leu Leu Tyr Tyr Tyr Val Phe

50

55

60

Val Ser Ser Asn Phe Asn Thr Val Lys Leu Leu Ser Phe Leu Ile Pro

65

70

75

80

Thr Gln Val Ala Tyr Leu Val Leu Gln Phe Asn Lys Cys Thr Val Tyr

85

90

95

Gly Asn Lys Ile Ile Lys Ile Asn Tyr Ser Leu Thr Ile Ile Cys Leu

100

105

110

Gly Val Thr Phe Leu Leu Ser Phe Pro Thr Met Leu Leu Thr Ile Leu

115

120

125

Phe Gly Ala Pro Leu Met Asp Leu Leu Trp Glu Thr Trp Leu Leu Ser

130

135

140

Leu His Phe Ala Phe Leu Ala Tyr Pro Ala Val Tyr Ser Val Phe Asn

145

150

155

160

Cys Asp Phe Lys Val Gly Leu Trp Lys Lys Tyr Phe Ile Phe Ile Val

165

170

175

1 2 2 / 1 6 1

Val Gly Gly Trp Ile Ser Cys Val Val Ile Pro Leu Asp Trp Asp Arg

180

185

190

Asp Trp Gln Asn Trp Pro Ile Pro Ile Val Val Gly Gly Tyr Leu Gly

195

200

205

Ala Leu Val Gly Tyr Thr Ile Gly Ala Tyr Ile

210

215

<210> 34

<211> 2493

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 34

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caagaccgtg atctccagcg cgataggccc ttccagaaat tgggtgtttgt gatcattgat 180

gctctcagat cagactttct ttttgattcg cagatttccc acttcaacaa cgtgcaccaa 240

tggctcaata cgggcgaagc atgggggttac acgtcatttg ctaatccgcc taccgtagcg 300

1 2 3 / 1 6 1

ctgcctagac tcaaaagtat tactacggga tctacaccta gcttcattga cttgctgctg 360

aatgtagccc aggacataga ttccaacgat ctttcggagc acgattcctg gctgcagcag 420

ttcatccaac ataataacac gattcgtttc atgggcgatg acacctggct gaaactgttc 480

ccacagcaat ggtttgactt cgctgacctg acacactcgt tctttgtcag tgatttcact 540

caagtcgata ataatgtgac gaggaacttg cccgggaaat tatttcagga atgggccag 600

tgggacgtgg ctatcctgca ttacttgggt cttgaccata tcgggcataa agatggcccc 660

cattcaaagt ttatggctgc taaacatcaa gaaatggaca gcattctgaa gtcaatatat 720

gatgaagtgt tggaacatga agatgacgat gatacactga tttgtgttct tggcgacat 780

ggaatgaacg aactgggcaa ccatggtggc tcttcagccg gcgaaacatc agcaggattg 840

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ttgcccatac acgctagtcc ggactggaat ttccagtatt tagagactgt tcaacaaatt 960

gatatcgtcc ccaccatagc agcactgttt ggtatgccaa tccccatgaa cagtgttggg 1020

ataataatac ctgacttttt acaactgttg cccaataagt tggcaagtat gaaagaaaat 1080

1 2 4 / 1 6 1

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gccgaagata ttatatacaa gatgtacact attcaagaaa cgtaaacc aa gtctgcaaca 1200

aattataatt atcctctttt gacactggct ttgtgtggtt tcctcataat aacaatcatc 1260

gccatttatg tattattaacg ttattctggg cctgattttt ggcagttgcg cgtttcttcc 1320

ctgtctgttc tgtagtttc cattatacta ggcgtttcca catttgcaag tagtttcatt 1380

gaagaggagc accaactgtg gtggtggata gtaactgcat tctcggcggt ccctctgttc 1440

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tcaatcaagt ttggaataa cagtggccag aaattcattt attctaactg tatgtccaat 1560

ctacttaatc agaatccttc ctggaagtgg tgcttaaata tgtagacatt tctagtgtg 1620

ataatggcat ctgctggttt tcaagtacta cttttattg tcactactat ttggtgggg 1680

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ctctttatgc atgatttact ggctaagata gactttgcac caactgaaag taacttgatt 1800

gtacttgcgc gcgttttctt ccaagcttgg gctattgttg tcatttcaag gttggtcctg 1860

1 2 5 / 1 6 1

acgaaattga aagtacttaa caagaactac ctcattaaag atatgaaagt ttatataaca 1920

attcttttga tgttccaaac ttcttctcag aacataggtc aatttctcgt tttccaaata 1980

ttagagtccc aaatttttta ctttttccaa aatattccaa cgcctcatt aacatcaaca 2040

agtaagattt atttttcgaa tttggtgtcc ttaattttac aaaattttac atttttccaa 2100

ttcgggtggca caaattccat ttctactata gaccttgga acgcatacca tgggtgttcc 2160

tcagactaca acatctacgt agtggggata ttaatgtccg ttgccaattt cgcgcoggca 2220

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ccaaaattat gttattttct tgggtggaat ttgtgatgg gattgctgaa tggctggta 2460

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<210> 35

<211> 830

1 2 6 / 1 6 1

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 35

Met Asn Leu Lys Gln Phe Thr Cys Leu Ser Cys Ala Gln Leu Leu Ala

1 5 10 15

Ile Leu Leu Phe Ile Phe Ala Phe Phe Pro Arg Lys Ile Val Leu Thr

20 25 30

Gly Ile Ser Lys Gln Asp Pro Asp Gln Asp Arg Asp Leu Gln Arg Asp

35 40 45

Arg Pro Phe Gln Lys Leu Val Phe Val Ile Ile Asp Ala Leu Arg Ser

50 55 60

Asp Phe Leu Phe Asp Ser Gln Ile Ser His Phe Asn Asn Val His Gln

65 70 75 80

Trp Leu Asn Thr Gly Glu Ala Trp Gly Tyr Thr Ser Phe Ala Asn Pro

85 90 95

Pro Thr Val Thr Leu Pro Arg Leu Lys Ser Ile Thr Thr Gly Ser Thr

100 105 110

Pro Ser Phe Ile Asp Leu Leu Leu Asn Val Ala Gln Asp Ile Asp Ser

1 2 7 / 1 6 1

115

120

125

Asn Asp Leu Ser Glu His Asp Ser Trp Leu Gln Gln Phe Ile Gln His

130

135

140

Asn Asn Thr Ile Arg Phe Met Gly Asp Asp Thr Trp Leu Lys Leu Phe

145

150

155

160

Pro Gln Gln Trp Phe Asp Phe Ala Asp Pro Thr His Ser Phe Phe Val

165

170

175

Ser Asp Phe Thr Gln Val Asp Asn Asn Val Thr Arg Asn Leu Pro Gly

180

185

190

Lys Leu Phe Gln Glu Trp Ala Gln Trp Asp Val Ala Ile Leu His Tyr

195

200

205

Leu Gly Leu Asp His Ile Gly His Lys Asp Gly Pro His Ser Lys Phe

210

215

220

Met Ala Ala Lys His Gln Glu Met Asp Ser Ile Leu Lys Ser Ile Tyr

225

230

235

240

Asp Glu Val Leu Glu His Glu Asp Asp Asp Asp Thr Leu Ile Cys Val

245

250

255

1 2 8 / 1 6 1

Leu Gly Asp His Gly Met Asn Glu Leu Gly Asn His Gly Gly Ser Ser

260

265

270

Ala Gly Glu Thr Ser Ala Gly Leu Leu Phe Leu Ser Pro Lys Leu Ala

275

280

285

Gln Phe Ala Arg Pro Glu Ser Gln Val Asn Tyr Thr Leu Pro Ile Asn

290

295

300

Ala Ser Pro Asp Trp Asn Phe Gln Tyr Leu Glu Thr Val Gln Gln Ile

305

310

315

320

Asp Ile Val Pro Thr Ile Ala Ala Leu Phe Gly Met Pro Ile Pro Met

325

330

335

Asn Ser Val Gly Ile Ile Ile Pro Asp Phe Leu Gln Leu Leu Pro Asn

340

345

350

Lys Leu Ala Ser Met Lys Glu Asn Phe Met His Leu Trp Lys Leu Ser

355

360

365

Asp His His Gly Glu Val Ala Leu Asp Asp Phe Thr Ala Glu Asp Ile

370

375

380

Tyr Thr Lys Met Tyr Thr Ile Gln Glu Thr Leu Thr Lys Ser Ala Thr

385

390

395

400

1 2 9 / 1 6 1

Asn Tyr Asn Tyr Pro Leu Leu Thr Leu Ala Phe Val Gly Phe Leu Ile

405

410

415

Ile Thr Ile Ile Ala Ile Tyr Val Leu Leu Arg Tyr Ser Gly Pro Asp

420

425

430

Phe Trp Gln Leu Arg Val Ser Ser Leu Ser Val Leu Leu Val Ser Ile

435

440

445

Ile Leu Gly Val Ser Thr Phe Ala Ser Ser Phe Ile Glu Glu Glu His

450

455

460

Gln Leu Trp Trp Trp Ile Val Thr Ala Phe Ser Ala Val Pro Leu Phe

465

470

475

480

Val Tyr Arg Leu Asn Val Leu Ile Ile Val Arg Trp Phe Ile Met Met

485

490

495

Ala Cys Val Arg Ser Ile Lys Phe Trp Asn Asn Ser Gly Gln Lys Phe

500

505

510

Ile Tyr Ser Asn Val Met Ser Asn Leu Leu Asn Gln Asn Pro Ser Trp

515

520

525

Lys Trp Cys Leu Asn Met Leu Thr Phe Leu Val Leu Ile Met Ala Ser

1 3 0 / 1 6 1

530

535

540

Ala Gly Phe Gln Val Leu His Phe Ile Val Thr Thr Ile Leu Val Gly

545

550

555

560

Leu Cys Phe Thr Tyr Lys Ile Ser Trp Glu Ile Val Asn Gly Asn Gln

565

570

575

Ala Glu Ile Pro Leu Phe Met His Asp Leu Leu Ala Lys Ile Asp Phe

580

585

590

Ala Pro Thr Glu Ser Asn Leu Ile Val Leu Ala Arg Val Phe Phe Gln

595

600

605

Ala Trp Ala Ile Val Val Ile Ser Arg Leu Val Leu Thr Lys Leu Lys

610

615

620

Val Leu Asn Lys Asn Tyr Leu Ile Lys Asp Met Lys Val Tyr Ile Thr

625

630

635

640

Ile Leu Leu Met Phe Gln Thr Ser Ser Gln Asn Ile Gly Gln Phe Leu

645

650

655

Val Phe Gln Ile Leu Glu Ser Gln Ile Phe Tyr Phe Phe Gln Asn Ile

660

665

670

1 3 1 / 1 6 1

Pro Thr Ala Ser Leu Thr Ser Thr Ser Lys Ile Tyr Phe Ser Asn Leu

675

680

685

Val Ser Leu Ile Leu Gln Asn Phe Thr Phe Phe Gln Phe Gly Gly Thr

690

695

700

Asn Ser Ile Ser Thr Ile Asp Leu Gly Asn Ala Tyr His Gly Val Ser

705

710

715

720

Ser Asp Tyr Asn Ile Tyr Val Val Gly Ile Leu Met Ser Val Ala Asn

725

730

735

Phe Ala Pro Ala Ile Tyr Trp Ser Met Leu Pro Trp Ser Ile Asn Tyr

740

745

750

Ala Ser Ile Pro Ala Gln Val Lys Leu Gln Thr Phe Ile Arg Ser Lys

755

760

765

Leu Pro Ala Phe Thr Tyr His Cys Ile Phe Gly Thr Cys Leu Met Thr

770

775

780

Ala Cys Val Val Leu Arg Phe His Leu Phe Ile Trp Ser Val Phe Ser

785

790

795

800

Pro Lys Leu Cys Tyr Phe Leu Gly Trp Asn Phe Val Met Gly Leu Leu

805

810

815

1 3 2 / 1 6 1

Asn Gly Trp Leu Pro Glu Leu Ala Leu Leu Cys Ala Leu Asp

820

825

830

<210> 36

<211> 1605

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 36

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tacattgagt cacttcaaaa taacaaattc caagatatc atctcgtaat accggtgtat 180

gttaagtcag atacttacag atttcctgac gtcatgacg ctatccaagt acaagttaac 240

catttattga attctcagga gcaacgggtc ccttggtctt tacaagttct tccatataat 300

gagactattg agcagatgga aagtgaaggc aaccagtttc atgtcgttac ttgaagtta 360

gacgaattta ttggttactc atcagcttac gacaccaaag aaacactagt atattacgac 420

gatgctgccg ttttaagtaa tgatctaccg tttttgttg ctcaaacatt ggtagagcac 480

1 3 3 / 1 6 1

actttccaat tggaatggac gcatttgaat aaaacgtgtg aaggcgtttc tacaacaac 540

gatgtogcaa tatcttatga tccaaacatt catttaagtg taactttatt gtcaggtgat 600

gggaatcctg ttgcatggga aattgagcct acattaactg actacttttc accttttagg 660

aagttcttat caccactggt aaattttaca gtagattcat ccattgttta tcataatgat 720

ttgaatttgc attcattaaa tggatcatgt acaagcgta cgtggtttga tctctctcat 780

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actcggattt cagacgaaat aaccacatta gattggaata gttatctagt tcctcaatgg 960

ggggttataa taataaataa aatgccgttg aagccaaatt cagtcattag cgaagattat 1020

ttagaacctt tgatgtaccg ttttgcgaca gatatttttc aactattggg attaacggag 1080

ggctcgcaag atttgttatc accttatatt accatagatt cattcaaaag gttgacaatt 1140

ttacagaatc tagataaagc tacggaaaca ttatggtcgt tagtgaaatt aactcaacaa 1200

tttcagggca tgtctatccc acgcgaagta tcggataatg ttatcgaagc tttagactta 1260

1 3 4 / 1 6 1

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aatgccctgc atctaagtaa tgaattggtt aaactatgcg aaaaggcatt tttcaatgga 1380

gaaatggttc aacaaaattt cttcccacaa gagcacatga tagctgtgta tttaccttta 1440

ttaggcccaa tatcggcagt catgttcttt ggtttctaca acgtgatgaa ggaaaagaat 1500

caaaagagta aaaagaatgg aaccgagaga gaagttgcta aagaaaaatt agagttgaaa 1560

gaggctcaaa aattacatgc tattgatggt gaagatgaat tatga 1605

<210> 37

<211> 534

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 37

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1

5

10

15

Ile Tyr Leu Phe Leu Gly Val Pro Leu Trp Tyr Lys Leu Thr Thr Val

20

25

30

1 3 5 / 1 6 1

Tyr Arg Ala Ser Leu Pro Ile Asn Tyr Ile Glu Ser Leu Gln Asn Asn

35

40

45

Lys Phe Gln Asp Ile His Leu Val Ile Pro Val Tyr Val Lys Ser Asp

50

55

60

Thr Tyr Arg Phe Pro Asp Val His Asp Ala Ile Gln Val Gln Val Asn

65

70

75

80

His Leu Leu Asn Ser Gln Glu Gln Arg Val Pro Trp Ser Leu Gln Val

85

90

95

Leu Pro Tyr Asn Glu Thr Ile Glu Gln Met Glu Ser Glu Gly Asn Gln

100

105

110

Phe His Val Val Thr Leu Lys Leu Asp Glu Phe Ile Gly Tyr Ser Ser

115

120

125

Ala Tyr Asp Thr Lys Glu Thr Leu Val Tyr Tyr Asp Asp Ala Ala Val

130

135

140

Leu Ser Asn Asp Leu Pro Phe Phe Val Ala Gln Thr Leu Val Glu His

145

150

155

160

Thr Phe Gln Leu Glu Trp Thr His Leu Asn Lys Thr Cys Glu Gly Val

165

170

175

1 3 6 / 1 6 1

Ser Thr Asn Asn Asp Val Ala Ile Ser Tyr Asp Pro Asn Ile His Leu

180

185

190

Ser Val Thr Leu Leu Ser Gly Asp Gly Asn Pro Val Ala Trp Glu Ile

195

200

205

Glu Pro Thr Leu Thr Asp Tyr Phe Ser Pro Phe Arg Lys Phe Leu Ser

210

215

220

Pro Leu Val Asn Phe Thr Val Asp Ser Ser Ile Val Tyr His Asn Asp

225

230

235

240

Leu Asn Leu His Ser Leu Asn Gly Ser Cys Thr Ser Val Thr Trp Phe

245

250

255

Asp Leu Ser His Thr Ile Asp Leu Ser Glu Leu Ser Ser Met Ala Tyr

260

265

270

Tyr Pro Glu Asp Ser Ala Leu Asn Leu Ala Ile Val Phe Pro Ser Ala

275

280

285

Ser Ser Ser Pro Asp Gly Leu Ala Phe Ile Asn Gly Thr Arg Ile Ser

290

295

300

Asp Glu Ile Thr Thr Leu Asp Trp Asn Ser Tyr Leu Val Pro Gln Trp

1 3 7 / 1 6 1

305 310 315 320

Gly Val Ile Ile Ile Asn Lys Met Pro Leu Lys Pro Asn Ser Val Ile

325 330 335

Ser Glu Asp Tyr Leu Glu Pro Met Met Tyr Arg Phe Ala Thr Asp Ile

340 345 350

Phe Gln Leu Leu Gly Leu Thr Glu Gly Ser Gln Asp Leu Leu Ser Pro

355 360 365

Tyr Ile Thr Ile Asp Ser Phe Lys Arg Leu Thr Ile Leu Gln Asn Leu

370 375 380

Asp Lys Ala Thr Glu Thr Leu Trp Ser Leu Val Lys Leu Thr Gln Gln

385 390 395 400

Phe Gln Gly Met Ser Ile Pro Arg Glu Val Ser Asp Asn Val Ile Glu

405 410 415

Ala Leu Asp Leu Arg Leu Gln Ile Ile Asp Leu Leu Asn Asp Pro Gly

420 425 430

Lys Gly Gly Asp Ile Val Trp Asn Asn Ala Leu His Leu Ser Asn Glu

435 440 445

i 3 8 / 1 6 1

Leu Val Lys Leu Cys Glu Lys Ala Phe Phe Asn Gly Glu Met Val Gln

450

455

460

Gln Asn Phe Phe Pro Gln Glu His Met Ile Ala Val Tyr Leu Pro Leu

465

470

475

480

Leu Gly Pro Ile Ser Ala Val Met Phe Phe Gly Phe Tyr Asn Val Met

485

490

495

Lys Glu Lys Asn Gln Lys Ser Lys Lys Asn Gly Thr Glu Arg Glu Val

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505

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Ala Lys Glu Lys Leu Glu Leu Lys Glu Ala Gln Lys Leu His Ala Ile

515

520

525

Asp Gly Glu Asp Glu Leu

530

<210> 38

<211> 1833

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 38

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60

1 3 9 / 1 6 1

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gtgttgaagc cgctgcccac caatgatctg ctactctcat ttgcattcca actgcaatcg 180

gaaccgtttg acccggccgt atcatccatg tcatatgatg cgtatgagca ctacacgact 240

ttcccacggg ccatcccacc attgttgga tctactgcca cgcgtcagtt tcatttaaga 300

tttaccagag gattctggga tgccctgtcg tggggacagt tgccacatgc tggaaaagag 360

gcaggtgcct caggtgtgga attgtggctg caagtgcagg ccatggatca ggaacaggcg 420

ttccataatt ggaaaaaact gtccaattca ttgagcggat tgttttgttc ttctttaaat 480

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atctgtaccg agaacttgac gccgttcata aaactattgc ctactagggg caaatccggt 660

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1 4 0 / 1 6 1

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1 4 1 / 1 6 1

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ggactcatat tcggtatgct atacaatttg atgggtgaaga gaatgggtcac cgtcgaagag 1740

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<210> 39

<211> 610

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 39

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1 5 10 15

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20 25 30

Trp Tyr Pro Tyr Asp Glu Ala Leu Val Leu Lys Pro Leu Pro Asn Asn

35 40 45

Asp Leu Leu Leu Ser Phe Ala Phe Gln Leu Gln Ser Glu Pro Phe Asp

1 4 2 / 1 6 1

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Phe	Pro	Arg	Ala	Ile	Pro	Pro	Leu	Leu	Glu	Ser	Thr	Ala	Thr	Arg	Gln
85	90	95													
Phe	His	Leu	Arg	Phe	Thr	Arg	Gly	Phe	Trp	Asp	Ala	Leu	Ser	Trp	Gly
100	105	110													
Gln	Leu	Pro	His	Ala	Gly	Lys	Glu	Ala	Gly	Ala	Ser	Gly	Val	Glu	Leu
115	120	125													
Trp	Ser	Gln	Val	Gln	Ala	Met	Asp	Gln	Glu	Gln	Ala	Phe	His	Asn	Trp
130	135	140													
Lys	Lys	Leu	Ser	Asn	Ser	Leu	Ser	Gly	Leu	Phe	Cys	Ser	Ser	Leu	Asn
145	150	155	160												
Phe	Ile	Asp	Glu	Ser	Arg	Thr	Thr	Phe	Pro	Arg	Arg	Ser	Tyr	Ala	Ser
165	170	175													
Asp	Ile	Gly	Ala	Pro	Leu	Phe	Asn	Ser	Thr	Glu	Lys	Leu	Tyr	Leu	Met
180	185	190													

1 4 3 / 1 6 1

Arg Ala Ser Leu Pro Asn Glu Pro Ile Cys Thr Glu Asn Leu Thr Pro

195

200

205

Phe Ile Lys Leu Leu Pro Thr Arg Gly Lys Ser Gly Leu Thr Ser Leu

210

215

220

Leu Asp Gly His Lys Leu Phe Asp Ser Leu Trp Asn Ser Ile Ser Leu

225

230

235

240

Asp Ile Ala Thr Ile Cys Ser Glu Asp Glu Asp Ala Leu Cys His Tyr

245

250

255

Glu Met Asp Ala Arg Ile Glu Met Val Thr His Val Pro Ser Ala Leu

260

265

270

Ala Arg Gly Glu Arg Pro Ile Pro Lys Pro Leu Asp Gly Asn Thr Leu

275

280

285

Arg Cys Asp Thr Asp Lys Pro Phe Asp Ser Tyr Gln Cys Phe Pro Leu

290

295

300

Pro Glu Pro Ser Gln Thr His Phe Lys Leu Ser Gln Leu Phe Ala Arg

305

310

315

320

Pro Ile Asn Asn Gly Asn Leu Phe Ala Asn Arg Pro Thr Arg Ile Cys

325

330

335

1 4 4 / 1 6 1

Ala Glu Val Asp Arg Ser Thr Trp Thr Ala Phe Leu Ser Val Asp Asp

340

345

350

Thr Ile Phe Ser Thr His Asp Asn Cys Phe Asp Leu Ser Asn Asp Gln

355

360

365

Asn Glu Gly Gly Ser Gly Tyr Asp Phe Ile Leu Glu Ser Thr Asp Thr

370

375

380

Thr Lys Val Thr Pro Ile Val Pro Val Pro Ile His Val Ser Arg Ser

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390

395

400

Leu Thr Gly Asn Gly Gln Asp Arg Gly Gly Met Arg Ile Val Phe His

405

410

415

Asn Asp Asn Asp Thr Pro Val Lys Leu Ile Tyr Phe Glu Ser Leu Pro

420

425

430

Trp Phe Met Arg Val Tyr Leu Ser Ser Leu Gln Ile Thr Ser Thr Thr

435

440

445

Ser Pro Gln Leu Gln Glu Asn Asp Ile Ile Leu Asp Lys Tyr Tyr Leu

450

455

460

Gln Ala Ala Asp Arg Lys Arg Pro Gly His Leu Glu Phe Thr Met Leu

1 4 5 / 1 6 1

465 470 475 480

Ile Pro Ala Asn Thr Asp Ile Val Met Thr Tyr Gln Phe Asp Lys Ala

485 490 495

Leu Leu Gln Phe Ala Glu Tyr Pro Pro Asp Ala Asn His Gly Phe Glu

500 505 510

Ile Asp Ala Ala Val Ile Thr Val Leu Ser Leu Glu Ser Ser Ser Ser

515 520 525

Leu Tyr Glu Met Arg Thr Ser Thr Leu Leu Leu Ser Leu Ser Thr Pro

530 535 540

Asp Phe Ser Met Pro Tyr Asn Val Ile Ile Leu Thr Ser Thr Ile Met

545 550 555 560

Gly Leu Ile Phe Gly Met Leu Tyr Asn Leu Met Val Lys Arg Met Val

565 570 575

Thr Val Glu Glu Ala Asp Lys Ile Thr Leu Gln Ser Gly Leu Lys Tyr

580 585 590

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595 600 605

1 4 6 / 1 6 1

Thr Asp

610

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<211> 1185

<212> DNA

<213> *Saccharomyces cerevisiae*

<400> 40

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acttcattta ggtcactaca ggaaggtata tacctactgc ggaacaacat ccaagtatat 180

aatcatgggg ttgttcacca tcctccaatt ttgatttttt ttctttccct ctttaattcc 240

gacaggttaa tttccctcat atacgcttta attgatggat taattgcgta tcagctgaca 300

gaggtaacaa aggctttcaa aaacttgaaa ctgaaagttt ggctacctgg acttctttat 360

gccgtgaatc ctttgaccct tttatcgtgc attagtcggt catcaatcat attcacaat 420

tttgctattt catcgtcatt gtattgcata ttagctgaag gaaacgttct tttgtcctct 480

1 4 7 / 1 6 1

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ttaggtatgc tgaaaagttg gaggcaaaga atattatctg ccattgtttc catactatct 600

ttattaattc tgctattatt cagctacagt atattaggca gccaaagttg gtcatttttg 660

acacaggttt atggatctat tataaccttt gagaaggttt ttccaaatct gggtttgtgg 720

tggtacttct tcattgaaat gtttgacacc ttcataccgt tcttcaaggc tgtattcaac 780

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<211> 394

<212> PRT

<213> *Saccharomyces cerevisiae*

<400> 41

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Ser Val Glu Phe Ser Thr Pro Val Thr Ser Phe Arg Ser Leu Gln Glu

35 40 45

Gly Ile Tyr Leu Leu Arg Asn Asn Ile Gln Val Tyr Asn His Gly Val

50 55 60

Val His His Pro Pro Ile Leu Ile Phe Phe Leu Ser Leu Phe Asn Ser

65 70 75 80

Asp Arg Leu Ile Ser Leu Ile Tyr Ala Leu Ile Asp Gly Leu Ile Ala

85 90 95

Tyr Gln Leu Thr Glu Val Thr Lys Ala Phe Lys Asn Leu Lys Leu Lys

100 105 110

1 4 9 / 1 6 1

Val Trp Leu Pro Gly Leu Leu Tyr Ala Val Asn Pro Leu Thr Leu Leu

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120

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Ser Cys Ile Ser Arg Ser Ser Ile Ile Phe Thr Asn Phe Ala Ile Ser

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Ser Ser Leu Tyr Cys Ile Leu Ala Glu Gly Asn Val Leu Leu Ser Ser

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Val Met Ile Ser Ile Ser Gly Tyr Leu Ser Val Tyr Pro Ile Leu Leu

165

170

175

Leu Ile Pro Leu Leu Gly Met Leu Lys Ser Trp Arg Gln Arg Ile Leu

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185

190

Ser Ala Ile Val Ser Ile Leu Ser Leu Leu Ile Leu Leu Leu Phe Ser

195

200

205

Tyr Ser Ile Leu Gly Ser Gln Ser Trp Ser Phe Leu Thr Gln Val Tyr

210

215

220

Gly Ser Ile Ile Thr Phe Glu Lys Val Phe Pro Asn Leu Gly Leu Trp

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230

235

240

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245

250

255

1 5 0 / 1 6 1

Ala Val Phe Asn Ile Phe Ile Ala Val Phe Ile Thr Pro Phe Thr Leu

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265

270

Arg Tyr His Lys Gln Pro Phe Tyr Ala Phe Ile Leu Cys Ile Gly Trp

275

280

285

Ile Val Leu Thr Lys Pro Tyr Pro Ser Leu Gly Asp Ala Gly Phe Phe

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Phe Ser Phe Leu Pro Phe Phe Thr Pro Leu Phe Gly Tyr Leu Arg Tyr

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Pro Ile Ile Ser Ala Leu Leu Phe Leu His Ala Ile Val Leu Ala Pro

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Ile Phe Tyr His Leu Trp Val Val Leu Gly Ser Gly Asn Ser Asn Phe

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Phe Tyr Ala Ile Ser Leu Val Tyr Ala Leu Ala Ile Ala Ser Ile Leu

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Val Asp Leu Asn Trp Ala Met Leu Arg Ile Glu Tyr Asp Asn Gly Ile

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<211> 255

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<213> Homo sapiens

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aagaaggctc agtga 255

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<211> 84

<212> PRT

<213> Homo sapiens

<400> 43

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20 25 30

Phe Ile Asp Ser Gln His Val Ile His Lys Tyr Phe Leu Pro Arg Ala

35 40 45

Tyr Ala Val Ala Ile Pro Leu Ala Ala Gly Leu Leu Leu Leu Phe

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Val Gly Leu Phe Ile Ser Tyr Val Met Leu Lys Thr Lys Arg Val Thr

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Lys Lys Ala Gln

<210> 44

<211> 369

<212> DNA

<213> Homo sapiens

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<211> 122

<212> PRT

<213> Homo sapiens

<400> 45

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Leu Leu Leu Arg Gly Ala Leu Leu Pro Ser Leu Ala Val Thr Met Thr

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Lys Leu Ala Gln Trp Leu Trp Gly Leu Ala Ile Leu Gly Ser Thr Trp

35

40

45

Val Ala Leu Thr Thr Gly Ala Leu Gly Leu Glu Leu Pro Leu Ser Cys

50

55

60

Gln Glu Val Leu Trp Pro Leu Pro Ala Tyr Leu Leu Val Ser Ala Gly

65

70

75

80

Cys Tyr Ala Leu Gly Thr Val Gly Tyr Arg Val Ala Thr Phe His Asp

85

90

95

Cys Glu Asp Ala Ala Arg Glu Leu Gln Ser Gln Ile Gln Glu Ala Arg

100

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Ala Asp Leu Ala Arg Arg Gly Leu Arg Phe

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120

<210> 46

<211> 744

<212> DNA

<213> Homo sapiens

<400> 46

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<211> 247

<212> PRT

<213> Homo sapiens

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Leu His Val Pro Cys Leu Lys Ile Leu Leu Ser Lys Gly Leu Gly Leu

35 40 45

Gly Ile Val Ala Gly Ser Leu Leu Val Lys Leu Pro Gln Val Phe Lys

50 55 60

Ile Arg Gly Ala Lys Ser Ala Glu Gly Leu Ser Leu Gln Ser Val Met

65 70 75 80

Leu Glu Leu Val Ala Leu Thr Gly Thr Met Val Tyr Ser Ile Thr Asn

85 90 95

Asn Phe Pro Phe Ser Ser Trp Gly Glu Ala Leu Phe Leu Met Leu Gln

100 105 110

1 5 7 / 1 6 1

Thr Ile Thr Ile Cys Phe Leu Val Met His Tyr Arg Gly Gln Thr Val

115

120

125

Lys Gly Val Ala Phe Leu Ala Cys Tyr Gly Leu Val Leu Leu Val Leu

130

135

140

Leu Ser Pro Leu Thr Pro Leu Thr Val Val Thr Leu Leu Gln Ala Ser

145

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155

160

Asn Val Pro Ala Val Val Val Gly Arg Leu Leu Gln Ala Ala Thr Asn

165

170

175

Tyr His Asn Gly Tyr Thr Gly Gln Leu Ser Ala Ile Thr Val Phe Leu

180

185

190

Leu Phe Gly Gly Ser Leu Ala Arg Ile Phe Thr Ser Ile Gln Glu Thr

195

200

205

Gly Asp Pro Leu Met Ala Gly Thr Phe Val Val Ser Ser Leu Cys Asn

210

215

220

Gly Leu Ile Ala Ala Gln Leu Leu Phe Tyr Trp Asn Ala Lys Pro Pro

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235

240

His Lys Gln Lys Lys Ala Gln

1 5 8 / 1 6 1

245

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<211> 25

<212> DNA

<213> Artificial

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<223> an artificially synthesized primer sequence

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25

<210> 49

<211> 30

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer sequence

<400> 49

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1 5 9 / 1 6 1

<210> 50

<211> 29

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer sequence

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<210> 51

<211> 30

<212> DNA

<213> Artificial

<220>

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<400> 51

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<210> 52

1 6 0 / 1 6 1

<211> 40

<212> DNA

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<211> 30

<212> DNA

<213> Artificial

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<210> 54

<211> 42

<212> DNA

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<213> Artificial

<220>

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<400> 54

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42

<210> 55

<211> 34

<212> DNA

<213> Artificial

<220>

<223> an artificially synthesized primer sequence

<400> 55

aggtaccgta cactccactc tatgatgatc attc

34

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(71) Applicant (for all designated States except US): **EI-SAI CO., LTD.** [JP/JP]; 6-10, Koishikawa 4-chome, Bunkyo-ku, Tokyo 112-8088 (JP).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HATA, Katsura** [JP/JP]; 20-6, Matsushiro 2-chome, Tsukuba-shi, Ibaraki 305-0035 (JP). **OGAWA, Kaoru** [JP/JP]; 102, 16-18, Kasuga 4-chome, Tsukuba-shi, Ibaraki 305-0821 (JP). **TSUKADA, Itaru** [JP/JP]; 7-4, Sakaecho 6-chome, Ushiku-shi, Ibaraki 300-1233 (JP). **NAKAMOTO, Kazutaka** [JP/JP]; 402, 15-8, Azuma 3-chome, Tsukuba-shi, Ibaraki 305-0031 (JP). **SAGANE, Koji** [JP/JP]; 303, 2-9, Amakubo 2-chome, Tsukuba-shi, Ibaraki 300-0005 (JP). **TANAKA, Keigo** [JP/JP]; 9-9, Matsushiro 2-chome, Tsukuba-shi, Ibaraki 305-0035 (JP). **TSUKAHARA, Kappei** [JP/JP]; 4-24, Ninomiya 4-chome, Tsukuba-shi, Ibaraki 305-0051 (JP). **HORII, Toshihiro** [JP/JP]; 19-2, Midorigaoka 3-chome, Toyonaka-shi, Osaka 560-0002 (JP).

(74) Agents: **SHIMIZU, Hatsushi et al.**; Kantetsu Tsukuba Bldg. 6F, 1-1-1, Oroshi-machi, Tsuchiura-shi, Ibaraki 300-0847 (JP).

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(84) Designated States (regional): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

Published:

— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

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(54) Title: METHODS OF SCREENING FOR COMPOUNDS THAT INHIBIT THE BIOSYNTHESIS OF GPI IN MALARIA PARASITES

(57) Abstract: The present inventors succeeded in isolating GWT1 (PfGWT1), which is one of the enzymes involved in GPI biosynthesis in the malaria parasite *P. falciparum*. In addition, the inventors revealed that degenerate mutant DNAs, with a lower AT content than the DNA encoding the PfGWT1 protein, can complement the phenotype of GWT1-deficient yeast. Based on the findings, the present invention provides the GWT1 protein of malaria parasites and the use of the protein in methods of screening for antimalarial drugs. The present invention also provides degenerate mutant DNAs encoding proteins involved in GPI biosynthesis, and which have a lower AT content than the original DNAs. The present invention also provides methods of screening for antimalarial drugs which use the degenerate mutant DNAs.



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INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP 03/14920

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N33/569 C07K14/445 C12N15/09

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HALL N ET AL: "Sequence of Plasmodium falciparum chromosomes 1, 3-9 and 13" NATURE (LONDON), vol. 419, no. 6906, 3 October 2002 (2002-10-03), pages 527-531, XP002277348 ISSN: 0028-0836 cited in the application & EMBL Entry: Q8ICL6 the whole document ----- -/--	1-4



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

20 April 2004

Date of mailing of the international search report

1.7.04

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Vogt, T

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No..
Y	GEROLD P ET AL: "BIOSYNTHESIS OF GLYCOSYLPHOSPHATIDYLINOSITOLS OF PLASMODIUM FALCIPARUM IN A CELL-FREE INCUBATION SYSTEM: INOSITOL ACYLATION IS NEEDED FOR MANNOSYLATION OF GLYCOSYLPHOSPHATIDYLINOSITOLS" BIOCHEMICAL JOURNAL, PORTLAND PRESS, LONDON, GB, vol. 344, 1999, pages 731-738, XP002935594 ISSN: 0264-6021 cited in the application the whole document	1-4,7-12
Y	WO 01/13923 A (DAVIDSON EUGENE A ;GOWDA D CHANNE (US); UNIV GEORGETOWN (US)) 1 March 2001 (2001-03-01) the whole document	1-4,7-12
Y	NAIK RAMACHANDRA S ET AL: "Developmental stage-specific biosynthesis of glycosylphosphatidylinositol anchors in intraerythrocytic Plasmodium falciparum and its inhibition in a novel manner by mannosamine" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 32, 11 August 2000 (2000-08-11), pages 24506-24511, XP002935593 ISSN: 0021-9258 the whole document	1-4,7-12
A,P	UMEMURA M ET AL: "GWT1 GENE IS REQUIRED FOR INOSITOL ACYLATION OF GLYCOSYLPHOSPHATIDYLINOSITOL ANCHORS IN YEAST" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 278, no. 26, 27 June 2003 (2003-06-27), pages 23639-23647, XP001179768 ISSN: 0021-9258 cited in the application the whole document	1-4
A	WO 02/04626 A (NAKAMOTO KAZUTAKA ;KAI JUNKO (JP); SAGANE KOJI (JP); TSUKADA ITARU) 17 January 2002 (2002-01-17) cited in the application	1-4
X	& EP-A-1300464 the whole document	5,6
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP 03/14920

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>GARDNER M J ET AL: "GENOME SEQUENCE OF THE HUMAN MALARIA PARASITE PLASMODIUM FALCIPARUM" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 419, 2002, pages 498-511, XP001156336 ISSN: 0028-0836 cited in the application the whole document</p>	1-4
A	<p>CARLTON J M ET AL: "GENOME SEQUENCE AND COMPARATIVE ANALYSIS OF THE MODEL RODENT MALARIA PARASITE PLASMODIUM YOELII YOELII" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 419, no. 6906, 2002, pages 512-519, XP001156232 ISSN: 0028-0836 cited in the application the whole document</p>	1-4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 03/14920

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee; this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14, 7-12

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: Claims 1-4 and 7-12.

Directed to DNA encoding a protein with the amino acid SEQ ID NO: 2 and 4, and methods of screening using said protein/DNA.

Invention 2: Claims 5 and 6.

Antimalarial drugs.

Invention 3: Claims 13-15 and 21-24 (partly).

DNA encoding a protein that has the activity of complementing the phenotype of a GPI synthase deficient yeast.

Invention 4: Claims 16-20 and 21-24 (partly).

DNA represented by SEQ ID NO: 5, vectors and transformants.

Invention 5: Claim 25.

A method of screening for antimalarial compounds using a GWT1 deficient fungus transfected with a DNA encoding for a malaria parasite GWT1 protein.

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0113923	A	01-03-2001	WO	0113923 A1	01-03-2001
WO 0204626	A	17-01-2002	AU	6947201 A	21-01-2002
			BR	0112263 A	24-06-2003
			CA	2415569 A1	17-01-2002
			CN	1449443 T	15-10-2003
			EP	1300464 A1	09-04-2003
			HU	0301657 A2	28-08-2003
			WO	0204626 A1	17-01-2002
			NO	20030045 A	06-03-2003
			US	2004038239 A1	26-02-2004